Genomic patterns of phenotypic adaptation in Italian wall lizard Podarcis siculus (Rafinesque-Schmaltz, 1810)

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Genomski obrasci fenotipske adaptacije primorske gušterice *Podarcis siculus* (Rafinesque-Schmaltz, 1810)

DOKTORSKI RAD

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This doctoral thesis was made in the Department of Biology, Faculty of Science, University of Zagreb, under the supervision of Assoc. Prof. Dr. Anamaria Štambuk, as part of the Postgraduate doctoral program in Biology at Faculty of Science, University of Zagreb.

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Doctoral thesis

GENOMIC PATTERNS OF PHENOTYPIC ADAPTATION IN ITALIAN WALL LIZARD *PODARCIS SICULUS* (RAFINESQUE-SCHMALTZ, 1810)

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Within a competitive exclusion experiment conducted in 1971, five pairs of *Podarcis siculus* lizards were transferred from islet Pod Kopište to a nearby islet of Pod Mrčaru in the Adriatic Sea. In only 35 years the newly established population exhibited significant morphological and ecological changes. This study combines experimental common garden approach with population genomics tools to discriminate between plastic and genomic response in Pod Mrčaru population. Crossing experiment in common garden pointed towards medium to high heritability of diverged phenotypic traits related to head shape, indicating they hold enough additive genetic variance to evolve under selection. Genotyping by sequencing elucidated genome-wide divergence between Pod Mrčaru and Pod Kopište populations. 18 loci putatively under selection were found to be associated with divergent phenotypic traits in source and newly established population, and/or differences in ecological variables among multiple *P. siculus* populations, suggesting rapid genomic adaptation on the islet of Pod Mrčaru.

(136 pages, 47 figures, 21 tables, 331 references, original in: English)

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GENOMSKI OBRASCI FENOTIPSKE ADAPTACIJE PRIMORSKE GUŠTERICE *PODARCIS SICULUS* (RAFINESQUE-SCHMALTZ, 1810)

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Sveučilište u Zagrebu

1971. godine pet pari gušterice *Podarcis siculus* je u sklopu istraživanja kompetitivne ekskluzije prebačeno s otočića Pod Kopište na susjedni otočić Pod Mrčaru u Jadranskom moru. U samo 35 godina nakon introdukcije novoustanovljena populacija je razvila značajne ekološke i morfološke razlike. Ovo istraživanje kombinira pokus zajedničkog okoliša s metodama populacijske genomike u svrhu razlučivanja između plastičnih i genomskih prilagodba u populaciji s otoka Pod Mrčaru. Eksperiment križanja u zajedničkom okolišu je ukazao na srednje visoku nasljednost fenotipskih obilježja vezanih uz oblik glave, što sugerira da sadrže dovoljno aditivne genetske varijabilnosti da evoluiraju pod utjecajem selekcije. Genotipiziranje putem sekvenciranja je pokazalo diferencijaciju na razini cijelog genoma između populacija s Pod Mrčaru i Pod Kopište. 18 lokusa identificiranih kao potencijalno pod utjecajem selekcije povezani su s divergirajućim fenotipskim obilježjima u ishodišnoj i novoustanovljenoj populaciji i/ili varijacijom u okolišnim čimbenicima među mnogobrojnim nativnim populacijama *P. siculus*, što ukazuje na brzu genomsku adaptaciju populacije na otoku Pod Mrčaru.

(136 stranica, 47 slika, 21 tablica, 331 literaturni navod, jezik izvornika: engleski)

Ključne riječi: *Podarcis siculus*, brza evolucija, zajednički okoliš, nasljednost, populacijska genomika, genomska adaptacija

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1. INTRODUCTION

In order to cope with swift changes in the environment, organisms need to be able to adapt to them quickly. This becomes especially relevant in wake of extreme climate shifts, extensive environmental modifications, and severe anthropogenic disturbances experienced by many ecosystems around the word today. Such large ecological changes impose strong selective pressure on adaptive phenotypes, which are expected to shift in distribution towards those that generate higher fitness in the new environment. Investigating the mechanisms driving this response in natural populations facilitates not only better understanding of the contemporary eco-evolutionary dynamics, but also the assessment of species persistence and adaptive evolution in the future.

Changes in adaptive phenotypic variation across habitats and populations can be the product of phenotypic plasticity (the ability of a single genotype to produce different phenotypes depending on the environmental context) and/or genomic adaptation (attributable to the sorting of standing genomic variation under natural selection). Phenotypic plasticity is one of the most common ways organisms cope with frequent environmental fluctuations, but it can also play a relevant role in species colonization and persistence in novel habitats (Hendry, 2017; Lande, 2015; Wang and Althoff, 2019). At the same time, natural selection acting on phenotypes important for fitness in the new environment increases the genome-wide abundance of favoured alleles, leading to adaptive genomic divergence (Endler, 1986; Levins, 1968; Nosil, 2012). In the conditions of strong selection, such evolutionary shifts can occur on ecologically relevant timescales, resulting in rapid evolution of phenotypically and genetically distinct populations in the course of only several generations (Carroll et al., 2007; Lescak et al., 2015; Marques et al., 2018; Stuart et al., 2014). Since plastic responses are not directly heritable, they appear to be most advantageous in short-term feedbacks, while genetic inheritance insures reliable transmission of the adaptive response across generations. However, relative contribution of phenotypic plasticity and genomic divergence to adaptation across spatial and temporal scales in natural populations is not clear. The two processes are frequently concurrent, with plasticity either promoting or constraining genomic adaptation (Auld et al., 2010; Lande, 2009; Oostra et al., 2018). Phenotype and genotype can further interact with environment in multifaceted ways, hindering the identification of specific mechanisms driving the evolutionary response to selection. Detecting evolution in the wild requires demonstrating that the phenotypic trait is variable (within and among populations),

and adaptive (i.e. improves fitness for individuals in different environments), that the observed variability has a genetic basis (i.e. is heritable), and that it promotes genomic divergence in trait-associated loci (irrespective of any neutral sources of variation) (Endler, 1986; Hendry, 2017; Pardo-Diaz et al., 2015). This can be notably difficult to achieve, as it calls for an extensive application of various experimental approaches, quantitative genetics modelling and modern population genomics techniques (Gienapp et al., 2017; Pardo-Diaz et al., 2015; Schlötterer et al., 2015). However, it is also the first step in inferring the evolutionally potential of contemporary populations and predicting their response to subsequent ecological change.

Adaptation processes can be difficult to observe and research in stable environments which experience little biotic or abiotic change, and are generally characterised by weak selection and populations that are well adapted to their habitat. In this respect, biological invasions oftentimes represent a good model system to study the basis of adaptive response in natural ecosystems. They are frequently well documented, enabling accurate measurement of the speed of phenotypic trait evolution, and can trigger remarkable phenotypic shifts in introduced and native populations alike (Cattau et al., 2018; Moran and Alexander, 2014; Rollins et al., 2015; Stuart et al., 2014). Indeed, one of the most intriguing examples of such rapid phenotypic adaptation comes from the deliberate introduction of the Italian wall lizard (*Podarcis siculus*) on a small islet of Pod Mrčaru in the Adriatic Sea off the cost of Croatia (Figure 1). As a part of competitive exclusion experiment conducted in 1971, 10 *P. siculus* individuals were transplanted from Pod Kopište Island to Pod Mrčaru, which was at the time inhabited by Dalmatian wall lizard, *Podarcis melisellensis* (Nevo et al., 1972). Merely 35 years after the introduction, *P. siculus* completely replaced the native *P. melisellensis* on Pod Mrčaru Island. More interestingly however, in the same time period the newly established population changed significantly in a range of morphological, physiological and ecological characteristics. The most striking of these is the occurrence of a new digestive organ in the introduced population – cecal valve, linked with the populations' shift in dietary preference towards herbivory (Herrel et al., 2008). Due to the remarkable magnitude and rate of the observed phenotypic shift supported by the known history of the introduction, this system provides an exceptional opportunity to study the mechanisms of rapid adaptation in the wild.

Figure 1 *Podarcis siculus* male on Pod Mrčaru Island.

1.1 Objectives and hypotheses

The main objective of this research is to explore the relative role of phenotypic plasticity and genetic adaptation in shaping the response to environmental change in Pod Mrčaru *P. siculus* population. To achieve this objective, three distinct hypotheses were tested:

H1: Phenotypic traits that show divergence between Pod Mrčaru and Pod Kopište *P. siculus* populations are heritable.

H2: Genome-wide differentiation exists between Pod Mrčaru and Pod Kopište *P. siculus* populations.

H3: Highly differentiated genomic markers between Pod Mrčaru and Pod Kopište *P. siculus* populations are associated with various phenotypic and/or environmental factors.

Specifically, this study aims to show that observed phenotypic evolution has a genomic background through the assessment of: **1)** heritable variation underlining rapidly diverging traits with the predictions that investigated traits possess enough additive genetic variance to evolve in response to selection, and that phenotypic patterns will persist when individuals are raised in common environment; **2**) combined effects of selection and genetic drift, which are

expected to result in genome-wide divergence between two focal populations; and **3)** adaptive role of a substantial number of highly diverged loci, with the prediction that those loci will be associated with phenotypic divergence or environmental variation in multi-population framework.

Heritable nature of diverged traits and adaptive role of diverged loci are highly indicative of rapid evolutionary response to changed ecological conditions.

1.2 Methodological overview

In order to test the first hypothesis (H1) and infer heritability patterns of putatively adaptive phenotypic traits in Pod Mrčaru lizards, a crossing experiment was set in a common garden. To that end, 100 adult *P. siculus* individuals were sampled from Pod Kopište and Pod Mrčaru islands and transferred to a common garden setting, where reciprocal crosses between and control crosses within populations were performed. Extensive data on 9 phenotypic traits of the head and body were obtained by photographing the individuals from successful pairings (62 F0 and 79 F1 individuals). Photographs were processed with image analysis software to obtain phenotypic measures of interest, using standard geometric morphometry based on landmark data.

Phenotypic variability among F0 and F1 individuals was first assessed using pairwise t-test or analysis of variance (ANOVA) and the principal component analysis (PCA) in order to analyse the persistence of phenotypic differences between individuals raised in the same environment. Varied set of quantitative genetics methods was then applied to the obtained dataset to estimate within-population heritability in traits of interest. First, traditional approaches of parent-offspring regression (PO regression) and ANOVA between full-siblings were applied to elucidate basic heritability patterns in analysed traits. Second, linear mixed model based on familial relationships (S.A.G.E., 2016), and animal models as implemented in restricted maximum likelihood (REML) software WOMBAT (Meyer, 2007) and Bayesian Markov chain Monte Carlo (MCMC) R package *MCMCglmm* (Hadfield, 2010; R Core Team, 2017), were employed to further partition the phenotypic variance into additive genetic and residual environmental component. Lastly, animal models were extended to account for other potential sources of non-genetic variance. Namely, influence of sex, experimental year, and parental source population was evaluated in order to exclude bias introduced by phenotypic similarity among individuals within specific groups.

Genotyping-by-sequencing (GBS) method was implemented to test the second hypothesis (H2) and examine the genomic differentiation in natural populations. Fourteen wild *P. siculus* populations, including Pod Mrčaru and Pod Kopište, and 12 *P. melisellensis* populations were genotyped (26 populations, 10-47 lizards per population and 600 individuals in total) and 19950 single nucleotide polymorphisms (SNPs) identified as common across all analysed populations and both species. Genomic diversity within the populations was described using statistical indices of allelic richness, expected and observed heterozygosity, and inbreeding coefficient. Next, genomic divergence between populations was estimated using F-statistics and analysis of principal components (PCA). Analysis of individual ancestry based on variational Bayesian inference was employed in software *fastStructure* (Raj et al., 2014) to further examine the genome-wide population structure.

Multiple outlier approaches were carried out on GBS data to test the third hypothesis (H3) and infer signatures of adaptive response in Pod Mrčaru *P. siculus* population. Three different genome scan methods were used to pinpoint outlier loci between two focal populations (Pod Kopište and Pod Mrčaru) and obtain a list of variant sites potentially under selection, each based on a discrete underlying algorithm: analysis of joint distribution of F_{ST} and heterozygosity from software Arlequin (Excoffier and Lischer, 2010); Bayesian F_{ST} outlier test based on Dirichlet-multinomial model for allele frequencies from BayeScan software (Foll and Gaggiotti, 2008); and a multivariate analysis of outlier loci with respect to population structure implemented in R package *PCAdapt* (Luu et al., 2017). Direct contribution of candidate loci identified as putatively under selection to genomic differentiation between two focal populations was verified using PCA analysis from R package *adegenet* (Jombart, 2008).

Genotype-phenotype-environment associations were employed to investigate the relationship between wild populations' genomic background, phenotypic characteristics, and local ecological conditions. Phenotypic differentiation (P_{ST} statistics, estimated for 14 morphological traits) was compared to genomic differentiation (F_{ST}) among populations in order to pinpoint traits showing higher divergence than would be expected under strictly neutral processes. Furthermore, latent factor mixed model (LFMM) regression analysis was implemented to investigate correlation between genotype and phenotype and correct for confounding effects due to population structure at the same time.

A set of 23 environmental variables was obtained from European Marine Observation and Data Network (EMODnet; Marine Information Service, 2017) and WorldClim (Hijmans et al., 2005) online databases for each sampled site and analysed for genotype-environment associations. Partial multivariate redundancy analysis (RDA) was employed on both neutral and putatively adaptive set of loci in order to partition the genomic variance between adaptive (ecological) and neutral (geographic isolation) components. Three different univariate Bayesian genome scans methods (Bayenv2 from Günther and Coop (2013), BayeScEnv from de Villemereuil and Gaggiotti (2015), and BayPass from Gautier (2015)) were further used to identify distinct changes in allele frequencies correlated with specific ecological variables.

Genomic regions of interest detected by genotype-phenotype-environment associations were compared to candidate loci for selection in order to pinpoint ecologically adaptive genomic markers in two experimental populations.

2. LITERATURE OVERVIEW

2.1 Phenotypic adaptation on contemporary timescales

Populations and species exist and thrive across a remarkably diverse range of ecosystems worldwide. In relatively stable environments populations are presumed to be well-adjusted to their habitat, and phenotype that allows for highest fitness is prevalent across most individuals. If the ecological conditions change, however, organisms must migrate, or respond adaptively to produce phenotypes better matched to the new environment. Individuals from the same ancestral population encountering different abiotic and/or biotic conditions may respond plastically and modify their phenotype to reach optimal fitness without change in their genomic background (Fox et al., 2019; West-Eberhard, 1989). Alternatively, distinct phenotypes may be favoured through selection and their association with genotype, leading to adaptive genomic divergence between affected populations (Levins, 1968; Nosil, 2012).

While historically their interaction was thought to be negligible across shorter time periods, today we know that evolutionary and ecological processes can occur on the same timescales (Carroll et al., 2007; Delong et al., 2016; Hairston et al., 2005; Rudman et al., 2017). Numerous and continuous interactions between the organism's phenotype, environment and performance are thus the drivers of contemporary eco-evolutionary dynamics (Hendry, 2017). An adaptive outcome of these interactions across the fitness landscapes is crucial if organisms are to persist and survive.

2.1.1 Phenotypic plasticity

Adaptive phenotypes can arise through phenotypic plasticity – the ability of single genotype to produce distinct phenotypes, across time and space. Plasticity is environmentally triggered, and has an important role in maintaining populations' fitness when ecological conditions change (Lande, 2014). Plastic responses thus seem to be most beneficial in more variable environments where the 'new' conditions are likely to be similar to those already experienced by previous generations (Hendry, 2017). However, plasticity might also play a significant part in biological colonisations, facilitating species spread, acclimatization and persistence in the novel environment (Davidson et al., 2011; Lande, 2015; Richards et al., 2006; Wang and Althoff, 2019). For instance, non-native *Podarcis muralis* shows phenotypic adaptation to colder climate by modifying embryonic incubation and developmental rates across its

expansion range (While et al., 2015). Similarly, *Anolis* lizards exhibit high phenotypic plasticity in the hind limb length, with the individuals raised on broad surfaces developing comparatively longer limbs then those raised on narrow perches (Losos et al., 2000). High levels of plasticity observed in this trait would have assisted the colonization of structurally diverse habitats during species' initial spread across the Caribbean (Kolbe et al., 2012; Losos et al., 2000).

Phenotypic plasticity is a ubiquitous physiological feature of organisms that can be observed across multiple molecular, physiological, morphological, behavioural and life-history levels. However, one of the most popularly studied mechanisms of plasticity today are epigenetic modifications (e.g. DNA methylation, histone modifications and chromatin remodelling, and RNA-mediated effects) that do not alter the DNA sequence, but can regulate phenotypic response through changes in gene expression. Epigenetic modifications can contribute to adaptation in wide variety of species and environments, with recent examples including acclimatization to fragmented and frequently disturbed habitat in *Arabidopis* (Schmid et al., 2018), marine and freshwater conditions in sticklebacks (Artemov et al., 2017; Heckwolf et al., 2020), and ocean acidification in reef-building corals (Liew et al., 2018). Moreover, despite the fact that most of the epigenome is reset during reproduction, it seems some epigenetic variation can be transmitted across generations (Duncan et al., 2014; Schlichting and Wund, 2014). Although transgenerational epigenetic inheritance is still a controversial topic (Heard and Martienssen, 2014; Horsthemke, 2018), it is well established that adaptive plasticity itself can have a genetic basis, evolve under selection, and promote further genomic adaptation (Chevin and Lande, 2011; Lande, 2014; Nussey et al., 2005).

Plasticity may influence evolutionary response by modifying selection strength on genotype, and promoting accumulation and the release of cryptic genetic variation (Draghi and Whitlock, 2012; Pfennig et al., 2010). In particular, selection acting on genomic variation underlying environmentally induced trait can both increase or decrease phenotypic plasticity through process known as 'genetic accommodation' (West-Eberhard, 2003). If the affected trait evolves decreased plasticity (i.e. environmental sensitivity) to the point that phenotype expression becomes independent of environmental stimulus, 'genetic assimilation' may occur (Waddington, 1953). In this case, plasticity enables the appearance of an adaptive environmentally-induced phenotype, and continued selection facilitates reconfiguration of genetic variation (i.e. 'canalization'), until the trait becomes 'fixed' or constitutively expressed in a population. Phenotypic plasticity may thus precede or even facilitate

evolutionary change (Pfennig et al., 2010; Pigliucci et al., 2006; Schlichting and Wund, 2014; Schneider and Meyer, 2017), although little is known about the exact mechanisms through which selection could promote genomic fixation of an initially non-heritable plastic trait.

While adaptive plasticity is relatively common, especially under environmental conditions that the population has experienced before, plasticity can also be neutral or even maladaptive, potentially constraining populations' adaptive potential (Ghalambor et al., 2007; Miner et al., 2005). Production and maintenance of plastic response can likewise be costly, particularly in the face of extreme environmental and phenotypic fluctuations, or ecological conditions that are rarely experienced (Auld et al., 2010; DeWitt et al., 1998; Hendry, 2017). Consequently, there are limits to what phenotypic plasticity can accomplish – plasticity alone may not be sufficient for a full adaptive response in strong environmental shifts like climate change, and some levels of phenotypic change are impossible to achieve through plasticity only (Duputié et al., 2015; Hendry, 2017; Phillimore et al., 2010; Van Buskirk et al., 2012).

2.1.2 Rapid genomic adaptation

Genomic changes are necessary for the phenotypic shift to occur outside the scope of plasticity, and an adaptive response to persist in the long term. Natural selection favours genotypes capable of producing phenotypes that show higher survival rate and reproduction success in the new environment. Modern synthesis emphasises this genetic foundation of evolution, predicting that adaptive genomic variants which allow higher fitness under selection will occur in greater frequency in a population over time (Mayr and Provine, 1980).

The common notion presumes that such evolutionary change is a slow and long process, with macro-evolutionary changes occurring over thousands of years to produce the remarkable biodiversity of species we witness today. However, growing empirical and theoretical evidence points towards adaptive evolution happening on ecologically relevant timescales (Carroll et al., 2007; Delong et al., 2016; Hairston et al., 2005; Lescak et al., 2015; Messer et al., 2016; Rudman et al., 2017). Such studies give examples of rapid or contemporary evolution, where, in the condition of strong selection pressure, genomic changes can lead to adaptive divergence among populations in only a couple of generations. Recent experimental evidence of rapid evolution include an 11-year long grassland biodiversity experiment indicating fast evolutionary response due to differential selection in monoculture and mixture environments (van Moorsel et al., 2019); a selection experiment demonstrating adaptive evolution from a highly derived stickleback ecotype in the course of only 19 years (Marques

et al., 2018); and a recent study showing that much of this genomic adaptation towards distinct stickleback ecotypes may occur in just one generation (Laurentino et al., 2020). Similar within-generation selection experiments were also used to demonstrate contemporary genome-wide allelic frequency changes that underlie adaptive divergence in two well investigated models of ecological speciation, *Rhagoletis* fruit fly (Egan et al., 2015) and *Timema* stick insects (Gompert et al., 2014). Experimental studies are further collaborated by fast-growing examples from the wild. Rapid evolution in a native lizard species after a competitor introduction to an island in Florida (Stuart et al., 2014), fast genomic adaptation to toxic pollution in wild Atlantic killifish populations (Reid et al., 2016), and to a novel host plant defence compounds in tobacco budworm (Fritz et al., 2017), as well as the rapid recent speciation observed in Darwin's finches from Galápagos Island of Daphne Major (Lamichhaney et al., 2018), are just some of the many instances of ecology driving contemporary evolutionary processes in natural populations.

Genomic adaptations can arise from new beneficial mutations or from standing genetic variation. The traditional population genetics model predicts either the appearance of a new advantageous mutation after the onset of selection, which then spreads through the population until it reaches fixation. This scenario leads to classic signature of 'hard' selective sweep, that is readily identified by the decrease in neutral variation at closely linked sites that hitch-hike to fixation alongside the beneficial mutation (Maynard Smith and Haigh, 1974). However, because most new mutations are non-adaptive, and it might take a long time for an advantageous variant to occur and spread across the population, adaptation from *de novo* mutations is considered relatively slow (Barrett and Schluter, 2008). In contrast, adaptation from standing genetic variation (or even recurrent mutation or migration in large populations, see Pennings and Hermisson (2006)) can lead to more rapid evolutionary shifts via so-called 'soft' sweeps (Hermisson and Pennings, 2017). Such sweeps are generally the product of pre-existing alleles that were neutral or even slightly deleterious before the onset of selection pressure, but increase in frequency towards fixation once they become adaptive due to ecological change (Hermisson and Pennings, 2005). On the other hand, while selective sweeps occur in a single or few loci of large individual effect, most complex phenotypic traits exhibit highly polygenic architecture, i.e. they are governed by multiple loci of small effect (Falconer and Mackay, 1996). Polygenic adaptation therefore describes a process in which selection acts on standing genomic variation at many loci simultaneously (Pritchard and Di Rienzo, 2010). In other words, when environmental change moves phenotypic optimum for a

quantitative polygenic trait, the population can adapt by small allele frequency shifts at many loci, which may or may not reach fixation. Multiple studies demonstrate that evolution can proceed rapidly by (re)using genomic variants already present in a population, although in such cases selection may be difficult to detect due to the absence of strong genomic signature (Crossley et al., 2017; Dayan et al., 2019; Hermisson and Pennings, 2017; Reid et al., 2016).

Adaptation in the wild is determined by various interactions between evolutionary forces of selection, mutation, recombination, gene flow and genetic drift, coupled with complex genomic architecture underlying quantitative traits. These processes are not mutually exclusive, which complicates investigations of molecular mechanisms underlying the genotype-phenotype-environment interactions that drive adaptive responses in nature (Messer et al., 2016). Gene flow, i.e. movement of individuals and their genetic material from one population to another, can limit local genomic adaptation by introducing maladaptive variants and offsetting allele frequency changes caused by selection (Lenormand, 2002). At the same time, however, gene flow may increase the pool of standing genetic variation that selection can act upon, and aid adaptation in small isolated populations (Frankham, 2015). Similarly, transmission of genetic variation between species by interbreeding (i.e. genetic introgression) can either impede or promote adaptation, depending on the fitness effect induced by new genetic material (Hedrick, 2013). On the other hand, bottlenecks (sharp reduction in effective population size as a result of severe ecological disturbance) and/or founder effects (bottleneck events attributable to small number of individuals establishing a new population) can reduce genomic variation in natural populations due to increase in genetic drift and inbreeding (Mayr and Provine, 1980). This is especially true for insular populations, where it can be difficult to disentangle signatures of recent directional selection and drift, which acts randomly across the genome. When investigating adaptation in natural conditions, it is therefore vital to differentiate between multifaceted ways evolutionary forces can shape genomic patterns in studied populations.

2.2 Heritability and the application of common garden experiments

Selection is the primary force generating evolutionary change, and heritable variation in phenotype is the raw material on which it acts (Endler, 1986; Mayr and Provine, 1980). Hence, the most straight forward way to determine the evolutionally potential of an adaptive trait is to evaluate its heritability. Variance in populations' phenotype (V_P) can be defined as the sum of genetic (V_G) and environmental variance (V_E), as well as various genotype-

environment associations ($V_{G\times E}$) (Falconer and Mackay, 1996; Lynch and Walsh, 1998; Rausher, 1992):

$$V_P = V_G + V_E + V_{G\times E} \qquad (1)$$

Genetic variance can be further separated to additive (V_A) and non-additive – dominance (V_D) and epistasis (V_I) component:

$$V_G = V_A + V_D + V_I \qquad (2)$$

Among these, additive variance has traditionally been of particular interest to evolutionary biologists and animal breeders. This is because in sexually-reproducing species, only additive variance is directly transmitted to the next generation, while dominance and epistasis relationships are created in the offspring anew (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Narrow-sense heritability (h^2) is therefore described as the ratio of additive genetic variance to the total phenotypic variance (to be distinguished from broad-sense heritability (H^2), which includes all levels of gene interactions, i.e. additive, dominance and epistasis) (Falconer and Mackay, 1996):

$$h^2 = \frac{V_A}{V_P} \qquad (3)$$

$$H^2 = \frac{V_G}{V_P} \qquad (4)$$

Importance of narrow-sense heritability for evolutionary studies becomes apparent when looking at the breeder's equation, in which the response to selection (R) is defined as narrow-sense heritability (h^2) of the trait multiplied by the strength of selection (S) on the trait, i.e. the higher the heritability of a trait, the higher its response to selection (Falconer and Mackay, 1996):

$$R = h^2 \times S \qquad (5)$$

Theory thus suggests that traits whose variance is to a great extent governed by genetic background will be highly hereditable, and able to evolve under selection. The variance in non-hereditable traits is, on the other hand, probably the result of phenotypic plasticity and developed under environmental or maternal effect. However, it is important to notice that while some phenotypic traits might exhibit low heritability, that does not mean they are not

associated with genetic components, or passed from one generation to next. This is notably the case for various fitness-related and/or life-history traits, which generally show low variability in a population at equilibrium, and thus low heritability estimates (because maladaptive variants are effectively eliminated through selection, e.g. see Kruuk et al., 2000; Mousseau and Roff, 1987; Price and Schulter, 1991). All traits have some genetic component, but the phenotypic variation of specific trait might be due to purely environmental factors – e.g. the fact that vertebrates have two eyes is very much genetically determined, but shows heritability close to zero because any phenotypic variance in a population is almost entirely due to environmental factors (accidents, for example). The opposite also holds true, if the traits show high additive genetic variance (i.e. high heritability estimates), that does not necessarily imply they cannot be shaped by environmental influence as well. Due to the direct influence of both genotypic and ecological component on phenotypic variation (Eq. 1), heritability estimates are population and environment specific, and cannot be extrapolated from one population or one environment to the other.

Traditional methods to estimate heritability are based on statistical associations between phenotypes of closely related individuals, i.e. parents and offspring in parent-offspring regression (PO-regression), and groups of siblings in ANOVA-based analyses. In PO-regression this amounts to estimating regression slope of offspring phenotype on mid-parent phenotypic value (Falconer and Mackay, 1996), while an ANOVA approach is based on the comparison of mean phenotype within and among groups of full or half-siblings (Falconer and Mackay, 1996; Lynch and Walsh, 1998). On the other hand, animal models can account for all levels of familial relatedness in complex pedigree structures, thus allowing for unbalanced designs and departures from strict assumptions characteristic for PO-regression and ANOVA approaches (Kruuk, 2004; Thomson et al., 2018; Wilson et al., 2010). Animal models are a form of mixed effects models – linear regressions that include both fixed (constants that affect the mean of a distribution) and random effects (variables whose levels are chosen at random from a larger population) as explanatory terms. In the simplest case, individual (i) phenotype (y) is modelled using fixed effect of population mean phenotype (μ), and individual breeding value (a_i) and residual error (e_i) as a random effect (Kruuk, 2004; Wilson et al., 2010):

$$y_i = \mu + a_i + e_i \qquad (6)$$

This allows for partitioning of phenotypic variance among random (additive and residual variance) components. However, animal models can be easily extended to account for other non-genetic factors that might influence phenotypic variation, e.g. age, sex, sampling year, and maternal or common environmental effects. As such, they are considered more robust and flexible in comparison with the traditional approaches aimed towards estimating additive genetic variance.

Over the years these quantitative genetic techniques have gradually broken out from their relatively limited use in long-lasting animal breeding designs requiring extensive pedigree collections and/or traditional laboratory model settings, to become widely employed in the studies of evolutionary processes in natural populations (Kruuk, 2004; Kruuk et al., 2008; Wilson et al., 2010). Nowadays, heritability studies are commonly used to deduce the relative contribution of phenotypic plasticity and additive genetic variation to adaptive population response in both the experimental settings (Bell et al., 2018; Jury et al., 2019; Logan et al., 2018), and in the wild (Carrete et al., 2016; Cattau et al., 2018; Gervais et al., 2020; Kimock et al., 2019). The experimental approaches typically rely on reciprocal transplant or common garden setting to account for phenotypic variation induced by environmental factors. While reciprocal transplant experiments test local adaptation by comparing fitness between native and introduced individuals, common garden experiments are specifically designed to study the genetic bases of variable phenotypic traits (de Villemereuil et al., 2015; Svensson et al., 2018). Common garden implies raising the individuals from different populations in the same environmental conditions, thus controlling for the effect of phenotypic plasticity and/or genotype-by-environment interactions. Beyond easier estimation of within-population heritability of phenotypic traits themselves, the persistence of phenotypic differentiation among individuals raised in the same environment may indicate that phenotypic differences between wild populations are heritable as well. Such approach thus enables testing for specific drivers of the observed phenotypic divergence – if environmental plasticity is significantly contributing to phenotypic differentiation between wild populations, those differences are expected to disappear when individuals are raised in the same environment. On the other hand, if selection is the one driving the divergent response, phenotypic differentiation is expected to hold even when individuals reared in the common garden. Subsequently, this allows for more straightforward partitioning between plastic responses and local adaptation in the wild (de Villemereuil et al., 2015), even in traits that are known to be highly heritable. When combined with modern population genomic methods, such

experimental approaches offer a powerful tool to study evolution in natural populations (de Villemereuil et al., 2015; Lepais and Bacles, 2014; Savolainen et al., 2013; Svensson et al., 2018).

2.3 Next-generation population genomics approaches

Methods of next generation sequencing (NGS) allow high-throughput parallel DNA sequencing, producing millions of reads (i.e. sequences) in one run, and at a relatively low financial cost (Davey et al., 2011; Stapley et al., 2010). This, in turn, facilitates detection of thousands of genomic markers, typically single nucleotide polymorphisms (SNPs), which can then be employed in modern population genomics studies. NGS methods provide information at higher resolution than can be obtained using traditional molecular genetics techniques, e.g. microsatellites, amplified fragment length polymorphism (AFLPs), allozymes, and mitochondrial DNA analyses (Angeloni et al., 2012; Davey et al., 2011; Seeb et al., 2011). Among various NGS techniques, reduced representation sequencing methods – namely restriction-site associated (RAD-Seq) and genotyping-by-sequencing (GBS) methods, have become a highly popular tool for inferring genome-wide patterns of local adaptation in natural populations (Baird et al., 2008; Elshire et al., 2011; Miller et al., 2007). These methods are not reliant on a large amount of genetic resources being developed in advance, and can be readily applied even in the absence of existing reference genome (i.e. whole genome assembled for the studied, or closely related species). As such, they are suitable for investigations in both model organisms used in typical genetic laboratory research, and non-model species which are more commonly in the focus of ecological studies in nature (Andrews and Luikart, 2014; Davey et al., 2011; Rochette and Catchen, 2017; Stapley et al., 2010).

Once obtained, genomic markers can be statistically analysed for those showing extremely low or high levels of differentiation among populations or ecotypes. This is usually accomplished using various genome scans, which assess allele frequencies among populations and identify those that show significant deviation from the distribution expected under strictly neutral model (Le Corre and Kremer, 2012; Nosil et al., 2009; Stapley et al., 2010). These ‘outliers’ are considered to be located in, or close to, quantitative trait loci (QTLs) that are adaptively diverging among analysed populations under directional selection. Outlier loci are commonly identified using various locus-specific F_{ST} methods (Antao et al., 2008; Excoffier and Lischer, 2010; Foll and Gaggiotti, 2008; Lotterhos and Whitlock, 2014).

However, drift can influence allelic distributions in a similar way to selection, and the robustness of the results may vary due to departures from the assumed demographic model. Hence, it is advisable to implement a multi-method approach, or estimate loci correlation with environmental or phenotypic variables in order to improve the confidence in outlier detection (de Villemereuil et al., 2014; Flanagan et al., 2018; François et al., 2016). Interactions between genomic markers and environmental factors can be analysed using genotype-environment association (GEA) methods. These studies enable identification of alleles showing strong shifts in frequencies based on changes in environmental factors, and give insight into the range of ecological pressures influencing patterns of genomic divergence in analysed populations (Benestan et al., 2016; Bernatchez et al., 2019; Forester et al., 2018; Jeffery et al., 2018; Rellstab et al., 2015). Methods exploring correlations between phenotypic variability and genomic divergence can give further insight into relative effect of putatively adaptive loci on trait modification (de Villemereuil et al., 2018b; Fuller et al., 2020; García-Navas et al., 2014). Such analyses can also provide groundwork for more detailed QTL mapping and genome-wide association (GWA) studies, which would allow for an in-depth characterisation of the genomic architecture underlying phenotypic traits of interest (Korte and Ashley, 2013; Savolainen et al., 2013; Stinchcombe and Hoekstra, 2008).

Advantages of employing modern population genomics methods study adaptation in the wild are thus multifold. They allow for: 1. detailed evaluation of genome-wide genomic diversity and differentiation; 2. identification of adaptive response in natural populations, 3. specification of genomic variants and regions under selection, and 4. an assessment of genotype association with phenotypic and environmental factors (Allendorf, 2017; Pardo-Diaz et al., 2015; Savolainen et al., 2013; Stapley et al., 2010). Hence, it’s not surprising that they became extremely prominent in contemporary studies of local adaptation, divergence and speciation. For instance, population genomics techniques have proven instrumental in uncovering molecular basis of adaptive divergence and early speciation scenarios between distinct ecotypes of *Timema* stick insects (Lucek et al., 2019; Riesch et al., 2017; Soria-Carrasco et al., 2014), stickleback fish (Jones et al., 2012; Lescak et al., 2015; Marques et al., 2018), and even the sympatric species of famous Darwin's finches (Chaves et al., 2016; Lamichhaney et al., 2015). Among reptiles, genomic approaches have mainly been used to study adaptation patterns in *Anolis* lizards, with recent examples including evolution in response to the invasion of a new competitor (Stuart et al., 2014), thermal adaptation due to mainland colonization (Campbell-Staton et al., 2016), extreme winter conditions (Campbell-

Staton et al., 2017), and urban habitats expansion (Campbell-Staton et al., 2020), as well as the altitude (Rodríguez et al., 2017) and geographic range (Prates et al., 2018) related genomic differentiation.

2.4 About the ambitious Italian wall lizard

Italian wall lizard (*Podarcis siculus*) is a robust species of lacertid lizards – adults usually have green and brown dorsal pigmentation pattern and can reach snout-vent length up to 9 cm, although morphology and coloration vary widely across regions, especially among insular populations (Arnold and Ovenden, 2002). It is primarily insectivorous, ground-dwelling and widely foraging species, showing high daylight activity during warmer parts of the year (Capula et al., 1993). Most individuals reach maturity after approximately one year in the wild, and adults exhibit strong sexual dimorphism – males are generally bigger and have larger heads than females. Like most reptiles, they are oviparous, laying multiple clutches with several eggs per season (Biaggini and Corti, 2019; Capula et al., 1993). *Podarcis siculus* can be found in various habitats – rocky coastal regions, shrublands, grasslands and forests, as well as the agricultural and urban areas. Its place of origin is Italian peninsula, but due to its opportunistic nature, apparent high adaptation potential, and frequent human-mediated introductions, today it's widespread across most of the Mediterranean coast (Arnold and Ovenden, 2002; Ilgaz et al., 2013; Podnar et al., 2005; Silva-Rocha et al., 2014, 2012), and has even reached all the way to Great Britain and USA (Behler and King, 1979; Conant and Collins, 1991; Kolbe et al., 2013).

Across its introduced range *P. siculus* is considered an invasive species as it quickly adapts to new environment and often displaces native lizard populations, reducing or taking over their habitat (Capula, 1992; D'Amico et al., 2018; Putman et al., 2020; Ribeiro and Sá-Sousa, 2018). As an ectothermic species, temperature has an extremely important influence in its biology and life history; however *P. siculus* appears to have acclimatized to broad range of environmental conditions across its wide distribution. Effective thermoregulation is achieved along its colonization range (Kapsalas et al., 2016), often through changes in seasonal and diet behaviour (Burke and Ner, 2005) or supercooling ability which provides tolerance to sub-zero exposures (Burke et al., 2002). Experimental evidence further suggests *P. siculus* can easily outcompete native species in the struggle for nutritional resources, as well as the prime hiding or basking spots (Damas-Moreira et al., 2020; Downes and Bauwens, 2004, 2002). For instance, introduction of *P. siculus* is thought to be the predominant reason behind

todays limited distribution and almost complete extinction of the critically endangered Aeolian wall lizard (*Podarcis raffonei*) (Capula, 2002). Cases of hybridization between *P. siculus* and other wall lizard species have also been reported, presenting an additional level of risk for endemic species along *P. siculus* expansion range. Hybridization with *P. siculus* has been documented for previously mentioned *P. raffonei* (Capula et al., 2002), as well as for Tyrrhenian (*Podarcis tiliguerta*) (Capula, 2002) and Sicilian wall lizard (*Podarcis wagleriana*) species (Capula, 1993).

Along Croatian coast and islands, *P. siculus* commonly occurs besides Dalmatian wall lizard, (*Podarcis melisellensis*). The species share many ecological traits and have similar life histories, however *P. siculus* appears to be more robust, generalist, and aggressive (Downes and Bauwens, 2002; Grbac and Brnin, 2006; Taverne et al., 2019). Although *P. siculus* and *P. melisellensis* are both found on some larger islands and in the mainland, two species are not syntopic, and are almost never found together on smaller islands (Gorman et al., 1975; Grbac and Brnin, 2006; Nevo et al., 1972; Podnar et al., 2005; Radovanović, 1959, 1956; Raynor, 1989). It is believed *P. siculus* spread to Croatian islands and coast by both land colonization and oversea introduction by man, and gradually replaced the native *P. melisellensis* on smaller islands along its expansion range by means of competitive exclusion (Podnar et al., 2005; Radovanović, 1965, 1959).

2.5 Lizards of Pod Kopište and Pod Mrčaru

In the last few decades, competitive exclusion between *P. siculus* and *P. melisellensis* along the Adriatic coast of Croatia has been investigated across various observational (Grbac and Brnin, 2006; Raynor, 1989) and experimental studies (Downes and Bauwens, 2002; Nikolić et al., 2019). However, groundwork for such research was laid even earlier, by a series of controlled experimental introductions conducted in the middle of 20th century. Radovanović (1965), who pioneered the theory of competitive exclusion between *P. siculus* and *P. melisellensis,* set up first recorded experimental introduction in 1958 when he introduced several *P. melisellensis* individuals onto the island of Koromašna, which was at the time inhabited by *P. siculus*. A year later he in turn introduced several *P. siculus* individuals on three islands inhabited by *P. melisellensis* (Mali Obrovanj, Dajnice, Krpeljina). Following Radovanović's research and finding inconclusive results, Nevo et al. (1972) set up an additional reciprocal transplant experiment in 1971, this time between two small islands in the Lastovo archipelago – Pod Mrčaru, at the time inhabited by *P. melisellensis*, and Pod

Kopište, inhabited by *P. siculus*. These two islets are situated at the distance of approximately 4 km, and due to the similarity in size, geology and general habitat, they provided an excellent setting for the designed experiment. Five pairs of *P. siculus* were transferred from islet Pod Kopište to islet Pod Mrčaru, and five pairs of *P. melisellensis* were in turn transplanted from Pod Mrčaru to Pod Kopište (Figure 2).

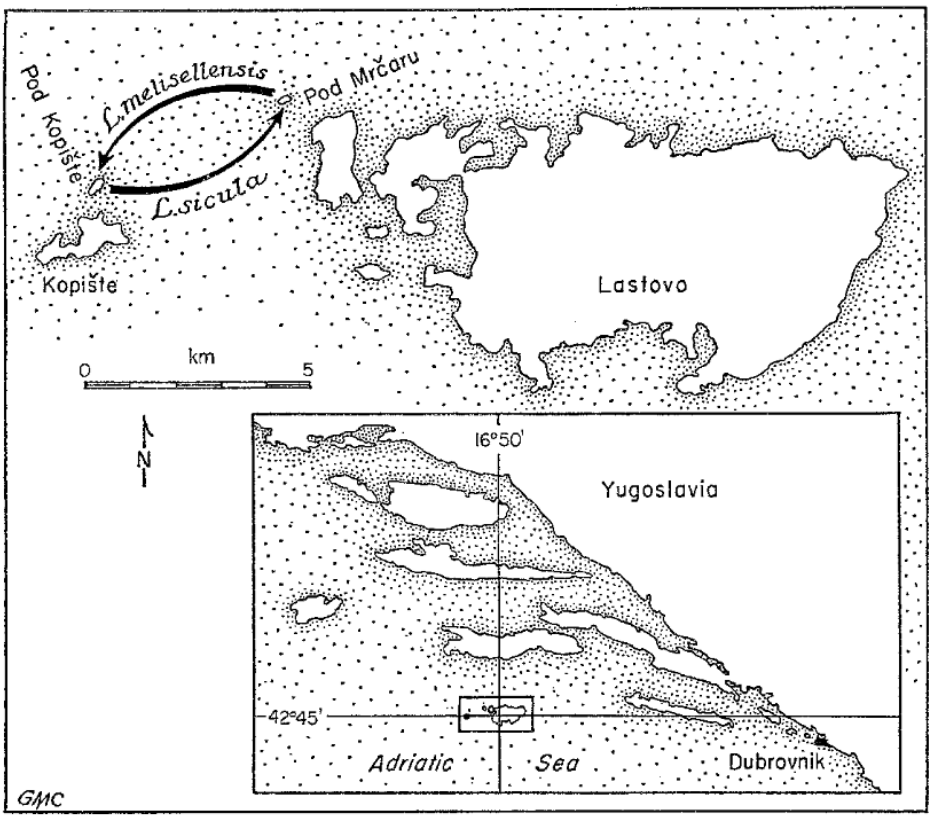


Figure 2 Map of the experimental *Podarcis* introductions in Lastovo archipelago. Reprinted with permission from Nevo et al. (1972), copyright Springer Nature.

Subsequent research conducted 35 years later showed that *P. siculus* completely replaced the native *P. melisellensis* on Pod Mrčaru Island, while no *P. melisellensis* individuals were found on the island of Pod Kopište (Herrel et al., 2008; Vervust et al., 2007). Analysis of mitochondrial DNA further suggested that the Pod Mrčaru *P. siculus* population is indeed descendent from the individuals transplanted from Pod Kopište, as no apparent genetic differentiation was found between lizards from the two islands (Herrel et al., 2008).This concluded the original competitive exclusion experiment, but the surveys exposed something even more interesting – in this short period of time the introduced *P. siculus* population on Pod Mrčaru changed their diet from predominantly insectivorous to omnivorous. Repeated analyses revealed both unusually high proportions of plant material in the stomach content,

and a remarkable occurrence of a completely new organ structure – cecal valve, in the hindgut of Pod Mrčaru lizards (Herrel et al., 2008; Taverne et al., 2019; Vervust et al., 2010).

Exploitation of a different dietary resource also appears to have facilitated a myriad other changes in head and body morphology, ecology, behaviour, and physiological performance in the introduced population (Herrel et al., 2008; Vervust et al., 2010, 2007). Indeed, most of the described phenotypic changes are reminiscent of the adaptations found in herbivorous species and can be directly connected to the observed shift towards plat-based diet in Pod Mrčaru population: 1. larger head, stronger bite force and changes in dentition allow easier mechanical fragmentation of fibrous plant material, 2. development of cecal valve slows down food passage, increases absorption surface, and allows them to acts as fermenting chambers, 3. presence of microbial endosymbionts that facilitate cellulose digestion, and 4. overall lengthening of the stomach and small intestine increases digestive efficiency (Herrel et al., 2008, 2004; Vervust et al., 2010; Wehrle et al., 2020).

While omnivory appears to be fairly common in lacertid lizards, especially in small insular populations, predominant herbivory is a relatively rare occurrence among reptiles in general (Cooper and Vitt, 2002; Espinoza et al., 2004; Herrel et al., 2004; Taverne et al., 2019; Van Damme, 1999). Notably, cecal valve are usually found exclusively in highly-specialized herbivorous lizards (Cooper and Vitt, 2002; Herrel et al., 2004; Iverson, 1982), and have been described in only one other lacertid species so far (Sagonas et al., 2015). Consequently, the development of such modifications in Pod Mrčaru lizard population over the course of only ~30 generations represents a unique example of rapid adaptation occurring before our very eyes. Plastic modifications of the digestive track features in response to dietary shifts were previously demonstrated in some reptile species (Hudson et al., 2018; Kohl et al., 2016; Starck et al., 2007; Starck and Beese, 2002), and some preliminary attempts have been made to study the mechanism of phenotypic response in Pod Mrčaru *P. siculus* population (Vervust et al., 2010). However, the relative role of phenotypic plasticity and genomic adaptation in the observed differentiation remains unknown. Further on, as no comprehensive population genomic studies have been conducted in this system so far, it is yet unclear how initial founder effect and subsequent phenotypic shift affected the underlying genomic patterns in the introduced *P. siculus* population.

3. MATERIALS AND METHODS

Licenses for sampling and handling animals in the field, as well experimental breeding in captivity were obtained from the Croatian Ministry of Environmental Protection and Energy.

3.1 Crossing experiment in the common garden

3.1.1 Sampling and experimental setup

In the early spring (March) of 2017 and 2018, *P. siculus* individuals were sampled in their natural environment on islets of Pod Mrčaru and Pod Kopište (Figure 2). The dates of field trip were chosen according to weather conditions and the onset of activity on islands in the given years. A total of 50 lizards were caught on each islet by use of a pole and a noose or by hand, sexed and transferred to the Zoological garden of Zagreb in individual bags to serve as parental generation in a crossing experiment in the common garden. Upon arrival to the zoo, male and female individuals were placed in separate terrariums for 4 weeks, with the aim of acclimatization and in order to make sure that caught females were not gravid. After the acclimatization period, controlled crossings were set within and between ancestral (Pod Kopište) and transplanted population (Pod Mrčaru) (Figure 3).

The pairs in the crossing experiment were kept in glass or plastic terrariums (60x30x30 cm) equipped with UV lamps (Arcadia T5 6% UVB Forest), small infra-red lamps, peat moss, rocks for perching and basking, dried bark, and plastic containers with vermiculite for hiding and laying eggs. Terrariums containing within and between island crosses were distributed randomly in the breeding facility. Terrariums were sprayed with water daily, and a small Petri dish containing drinking water was checked and refilled according to consumption. The room was subjected to the same light regime (12L:12D) and constant temperature (23–24 °C diurnal, 20 °C nocturnal). During daylight period lizards were able to thermo-regulate by repositioning themselves respectively to the infra-red light. Individuals were kept on the same cricket-based diet – 1 or 2 small to medium sized crickets (*Gryllus assimilis*), periodically covered in calcium supplement (fine dust JBL MicroCalcium), were given to each individual depending on its size three times a week. In mid-December, temperature was gradually decreased to ~12 °C, light regime modified to 9L:15D, and feeding interrupted to induce hibernation which lasted approximately three months (until mid-March). In this period all activities were paused to be resumed once the feeding, light and temperature regimes were re-established.

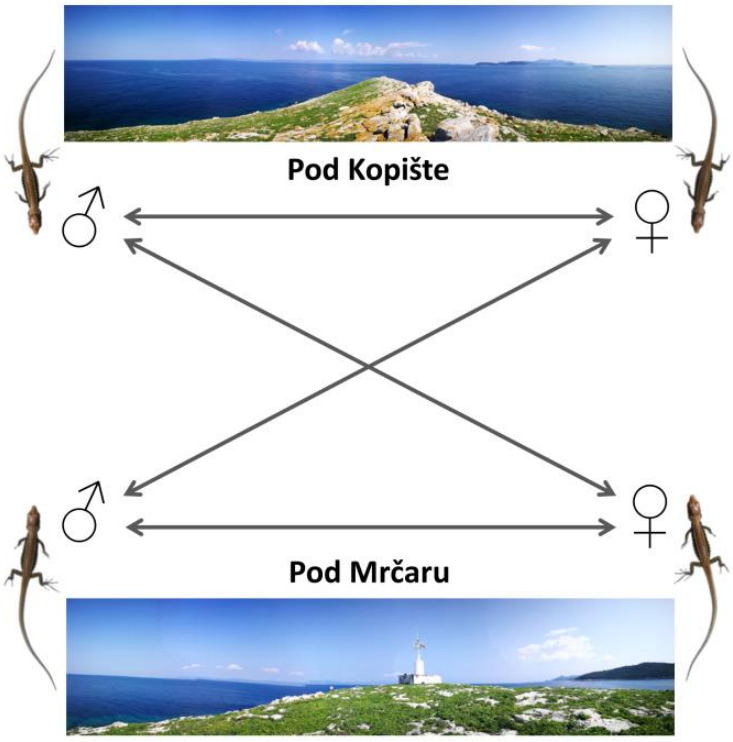


Figure 3 Scheme of the crossing experiment between *P. siculus* individuals sampled from Pod Kopište (PK) and Pod Mrčaru (PM) population. Controlled crossings were set for each pair of island combinations (PK♂-PK♀, PK♂-PM♀, PM♂-PK♀, and PM♂ -PM♀).

Female abdomens and terrariums were checked daily during mating season and weekly out of mating season for mating scars and laid eggs. When an egg was found, it was placed separately from the parents in a closed plastic container (100 ml; 2/3 filled with moist vermiculite), and kept in an incubator at constant temperature of 28–29 °C. After hatching, offspring were placed in individual terrariums distributed randomly through the room. Offspring were raised under the same conditions their parents were kept in and fed with the same cricket species but much smaller in size.

All individuals were marked with color-coded visible implant fluorescent elastomer tags (Northwest Marine Technologies) for their reliable identification. The experiment was conducted in two consecutive years to increase the number of reared families and offspring, and the substrate used for egg incubation changed to larger grain vermiculite in 2018 to improve egg hatching success. A piece of tail tissue was taken from all individuals in common garden experiment and stored in 96% ethanol for genomic analyses.

3.1.2 Phenotypisation

In order to determine their phenotype, a set of morphological measures was extensively collected for all the individuals from the crossing experiment. The parental generation was phenotyped after the mating period in each respective year in order to not disturb the premating behaviour, and the offspring the day after they hatched and every two months afterwards until reaching approximately 18 months of age. Morphological measures were collected by photographing the individuals from the dorsal side of the body and left lateral side of the head. For the photographing, lizards were placed as flat as possible on a horizontal surface with a graph paper in the background to ensure proper scaling. All pictures were taken with the camera (Canon EOS 450D, with Canon EF-S 18-55mm f lens) placed perpendicular to the paper, at the same distance from the object (16 cm), and using the same camera settings (automatic mode with zoom set to 18 mm for dorsal and to 35 mm for lateral pictures of the head).

The images were analysed with image analysis software ImageJ (Schneider et al., 2012) to obtain morphological measures of interest. Dorsal images of lizards and tools available in ImageJ software were used to calculate phenotypic measures of head width (HWdth), body length to the hip (LtHip) and tail length (TailL) (Figure 4A). Each measurement was taken three times independently and averaged. The averaged value was used in all posterior analysis. Using ImageJ software, eight landmarks were placed at the specific points on the left lateral pictures of the head (Figure 4B). The coordinates of those landmarks were then exported and analysed with custom made script in R v.4.0.0 (R Core Team, 2017) in order to measure the exact distance between them. Five morphometric measurements were obtained from the eight landmark coordinates: head height (HHght), head length (HLgth), snout length (SnLgh), lower jaw length (LwJaL), and lower jaw outlever (LwJaO) (Figure 4B). This process was repeated three times independently, the three values for each measurement were averaged, and the averaged value was used in all posterior analysis.

To assess repeatability of the employed phenotypisation method (photographing and image analysis), 21 F1 individuals were re-phenotyped in October 2020. Three separate photographs were taken for each individual and each photograph was in turn analysed three times independently, obtaining 9 replicate measures for each phenotypic trait. Repeatability was estimated using one-way analysis of variance (ANOVA) approach described in Arnqvist and Mårtensson (1998). Repeatability estimates obtained using this method range from 0 to 1

where, for instance, repeatability of 0.8 indicates that 80% of the total phenotypic variation is attributable to variation that is naturally present between analysed individuals, while the remaining 20% of phenotypic variation is attributable to differences among technical replicates.

Additionally, bite force was measured for all individuals used in the crossing experiment, using a Kistler force transducer set in a custom-built holder and connected to a Kistler charge amplifier. The bite force of 2017 parental generation was measured in the time outside of the breeding period and of juveniles when they were approximately four-months old. Bite force was measured 3 times and the maximum recorded value was taken into the account. This maximum value was additionally multiplied by 0.67 before further analysis in order to correct for the lever arm length. Unfortunately, bite force measure was not obtained from additional F0 individuals added to the experiment in 2018 and bite force analyses were therefore preformed on a smaller pedigree dataset.

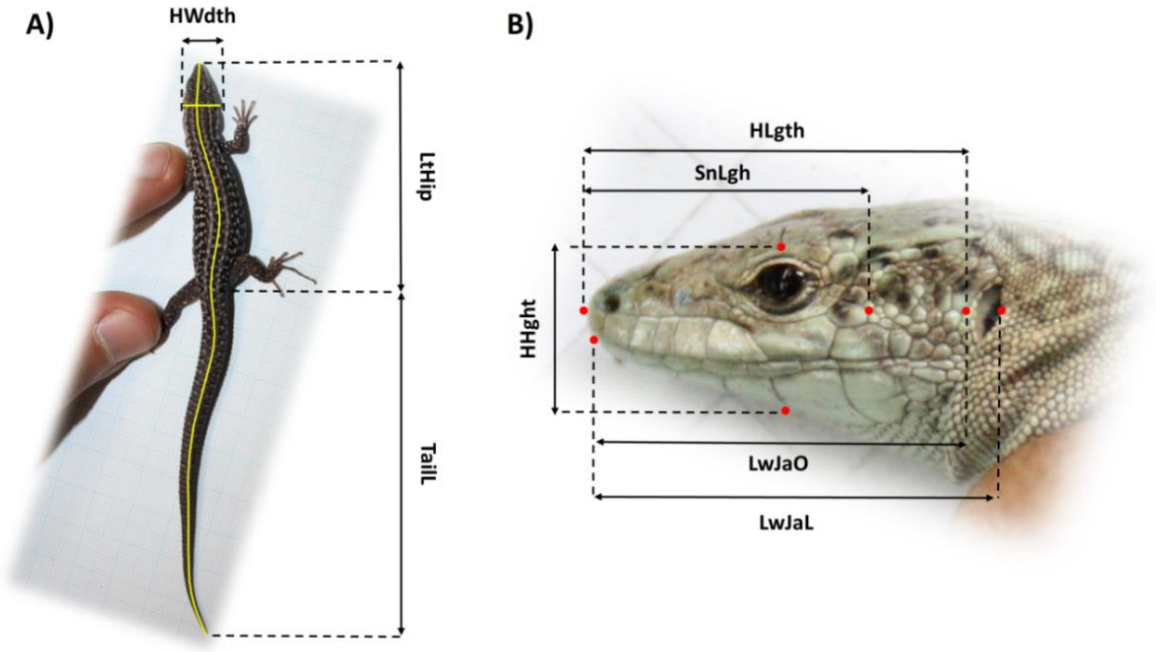


Figure 4 Morphological measures collected from photographs of **A)** dorsal side of the body, and **B)** lateral side of the head of the lizards used in the crossing experiment. Abbreviations are defined in the text.

3.1.3 Statistical analyses and heritability estimation

All statistical analyses were conducted in R v.4.0.0 (R Core Team, 2017). Phenotypic measurements were size-corrected using linear regression on length to hip (LtHip) to eliminate variation resulting from allometric growth. Absolute minimum value was then increased by 0.01 and added to regression residuals to account for negative values, and a power of two transformation applied to in order to approximate normal distribution as judged by Shapiro-Wilk test and the empirical distribution observed in a skewness-kurtosis plot (Cullen and Frey, 1999) from package *fitdidtrplus* v.1.1.1 (Delignette-Muller and Dutang, 2015). Bite force residuals were not transformed in any way, and raw length to hip was first scaled and then arcsine transformed. All measurements were treated as Gaussian in posterior analyses.

Variability in offspring phenotype was examined using an analysis of variance (ANOVA), followed by Tukey's honest significant difference test, in order to investigate specific differences between offspring groups. A pairwise t-test was used to examine the variability in parental phenotype. Principal component analysis (PCA) was run using only head size traits to estimate body-size-independent variation in head morphology among experimental individuals. PCA scores were further analysed using generalized linear models (GLM) fitted in ANOVA to test the significance of group specific separation in multivariate phenotype. Because *P. siculus* individuals generally exhibit high sexual dimorphism in most of the analysed traits (Taverne et al., 2019), males and female offspring were analysed separately. All analyses were conducted using functions available in R package *stats* (R Core Team, 2017).

Trait heritability was estimated from obtained pedigree data using several different approaches in order to evaluate evolutionary potential of examined traits in wild populations. Analysis of variance among full-sibs (ANOVA) and parent-offspring (PO) regression were performed in R using *stats* v.3.6.2 package. Heritability and standard error were calculated from the ANOVA output using major steps described in (Roff, 1997). Mid-parent (average phenotypic value of the two parents) and mean offspring (average phenotypic value across all offspring from one family) values were used to analyse phenotypic variability among different families in PO regression. Heritability was considered equal to the slope of the regression, and standard error of heritability estimation to the standard error of the slope (Roff, 1997). Another linear regression model, which allows for partitioning of phenotypic

variance to polygenic and individual random component after regressing on covariates, was employed using ASSOC (Bochud, 2012; S.A.G.E., 2016). Heritability was estimated from the nuclear family dataset after applying a George and Elston transformation to the phenotypic variables. Univariate animal models, as implemented in REML WOMBAT (Meyer, 2007) and Bayesian MCMCglmm (Hadfield, 2010), were used to further assess additive genetic variance underlying phenotypic traits of interest. WOMBAT was run with default settings under the average information (AI) algorithm. For MCMCglmm models, a weakly informative inverse gamma prior was specified, total number of iterations set to 2,500,000, with burn-in period of 500,000 and thinning interval of 100. Model outputs were checked for lack of convergence, inadequate effective sample sizes and/or high levels of autocorrelation. In analyses where negative values of heritability were obtained, estimates were assumed to be zero and were expressed as such.

Several factors were identified as potential sources of non-genetic phenotypic covariance in analysed pedigree – namely sexual dimorphism, and differences in year of birth (2017 – 2018 F1 and F0 cohort) or parental origin. Significance of those factors was tested by including them as covariates in ASSOC and as fixed effects in WOMBAT and MCMCglmm models in a step-by-step procedure. Models with and without the effect were examined by comparing maximum log likelihoods (ASSOC), and testing them against a chi-square distribution with one degree of freedom (WOMBAT), or comparing deviance information criterion (DIC) metric (MCMCglmm). Due to the relatively small sample size it was not possible to significantly estimate effect of sex, experimental year or source population using the two traditional approaches of PO-regression and ANOVA.

3.2 Data and sample collection from wild populations

3.2.1 DNA sampling and phenotypisation

14 populations of Italian wall lizard (*P. siculus*), and 12 populations of Dalmatian wall lizard (*P. melisellensis*) were used to study the genomic patterns of adaptive divergence in the wild (Figure 5). The morphological data and DNA samples of individuals from 20 of those populations (including Pod Kopište and Pod Mrčaru) were sampled in 2016 and provided for this study by Dr. Anthony Herrel from French National Centre for Scientific Research (CNRS). Additional 6 *P. siculus* populations were sampled in 2019 – Veliki Dupinić (DU), Kluda (KL), Obrovanj (OB), Oštrica (OS), Rakita (RK), and Visovac (VC).

Lizards were caught by use of poles with nooses at the end, or by hand, and sexed. A set of morphological measures of the body was taken for each individual to the nearest 0.01 mm with a digital calliper (Powerfix Profi+). Body mass was determined using an analogue scale, and bite force measured using a Kistler force transducer and charge amplifier. Bite force was measured 5 times and the highest recorded value was used in the posterior analysis. Bite force measure was further multiplied by 0.67 to correct for lever arms. In total, 14 morphometric measurements (Table 1) were considered in the phenotypic analysis of wild populations. A piece of tail tissue was taken and stored in 96% ethanol for genomic and phylogenetic (Supplementary material, Table S1) analyses. After the data collection, lizards were returned to their natural habitat.

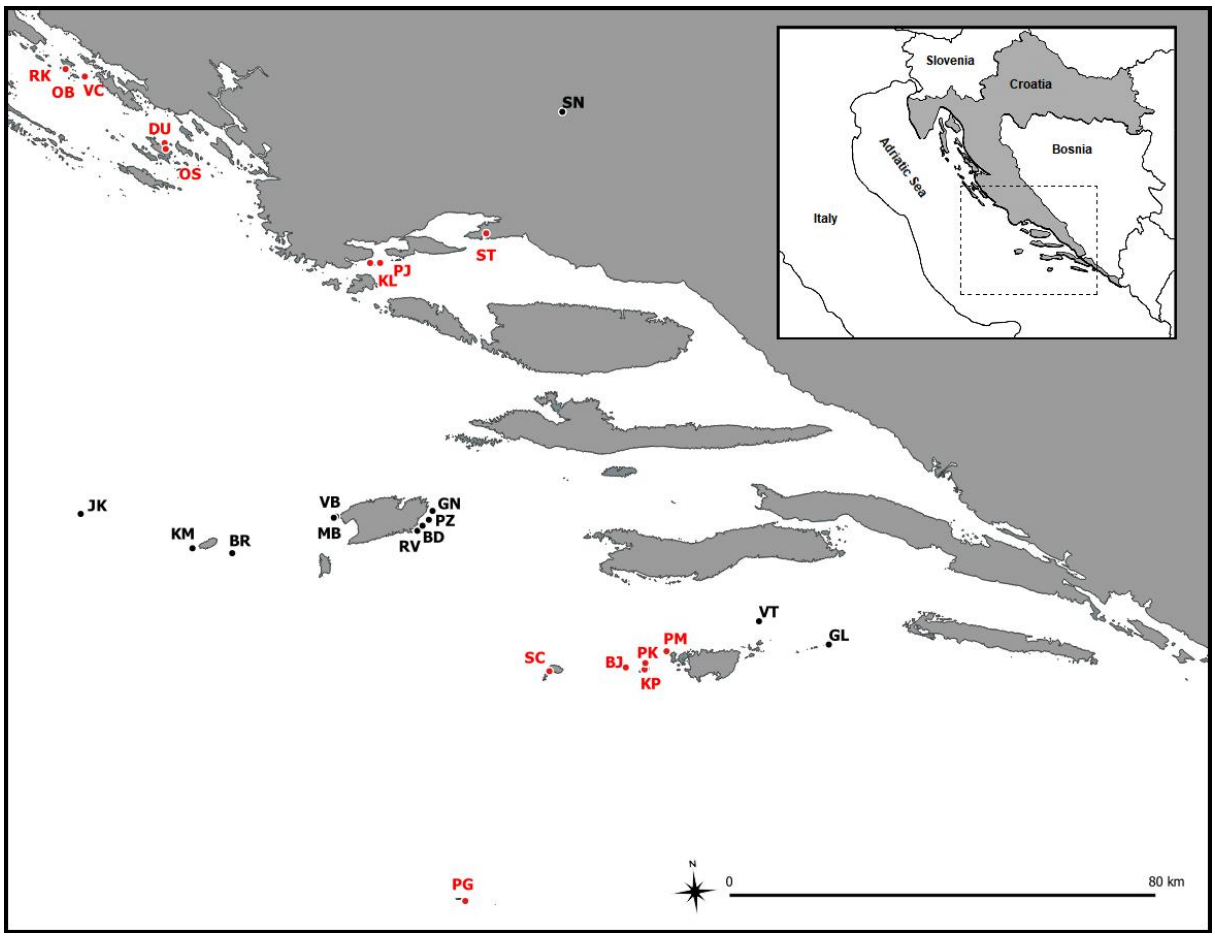


Figure 5 Map of the sampling locations of wild *Podarcis* populations. *P. siculus* populations are marked in red (ST – Split, PJ – Pijavica, SC – Sušac, BJ – Bijelac, KP – Kopište, PK – Pod Kopište, PM – Pod Mrčaru, PG – Mala Palagruža, RK – Rakita, OB – Obrovanj, VC – Visovac, DU – Veliki Dupinić, OS – Oštrica, KL - Kluda) and *P. melisellensis* in black color (SN – Sinj, JK – Jabuka, KM – Kamik, BR – Brusnik, MB – Mali Barjak, VB – Veli Barjak, RV – Ravnik, BD – Veli Budikovac, PZ – Mali Paržanj, GN – Grebeni, VT – Veli Tajan, GL - Glavat).

Before employing morphometric data for assessments of populations' phenotypic differentiation and genotype-phenotype interactions, raw measurements were size corrected, using linear regression implemented in R *stats* v.3.6.2 package and snout-vent length (SVLgh) measure as the regressor. Datasets collected in 2016 and 2019 were size-corrected separately to account for putative inter-observer effect and seasonal variation in phenotype. Since data for all traits was either normally distributed, or showed close to normal distribution in bootstrap simulations (as judged by Shapiro-Wilk test and Cullen and Fray graph), regression residuals were not transformed any further before subsequent analyses.

Table 1 Morphometric measures included in the phenotypic analysis of *P. siculus* and *P. melisellensis* wild populations.

ID	Measurement	Description
SVLgh	Snout-vent length	Measured from the tip of the snout to the posterior edge of the anal scale (mm)
HLgth	Head length	Measured from the from the tip of the upper jaw to the back of the parietal bone (mm)
HWdth	Head width	Measured at the widest part of the head (at the level of jugal bones) (mm)
HHgth	Head height	Measured at the highest part of the head (posterior to the orbits) (mm)
LwJaL	Lower jaw length	Measured from the tip of the lower jaw to the back of the articular process (to the to the posterior edge of the ear opening) (mm)
LwJaO	Lower jaw outlever	Measured from the tip of the lower jaw to the posterior edge of the quadrate (anterior edge of the ear opening) (mm)
SnLgh	Snout length	Measured from the tip of the lower jaw to the coronoid (posterior edge of the jugal) (mm)
ILLgh	Interlimb length	Measured as the distance between the points of insertion of the fore and hind limbs (mm)
HLLgh	Hind limb length	Cumulative length of femur, tibia, metatarsus and 4th hind toe (mm)
FLLgh	Front limb length	Cumulative length of humerus, radius, metacarpus and 3rd forward toe (mm)
BHgth	Body height	Measured at the highest part of the body (mm)
BWdth	Body width	Measured at the widest part of the body (mm)
BiteF	Bite force	Maximum value across five consecutive measurements (kN)
BMs	Body mass	Measured to the nearest 0.1 mg

3.2.2 Environmental and geographical variable collection

In order to characterize the habitat of each studied natural population, interpolated values of 23 different environmental parameters (Table 2) for all sampled sites were obtained from European Marine Observation Data Network (EMODnet) Bathymetry portal (Marine Information Service, 2017) and WorldClim (Hijmans et al., 2005) database.

Table 2 List of environmental variables used in the analysis.

Variable	Description
Geographical variables	
Longitude	Geographic longitude
Latitude	Geographic latitude
Ecological variables	
Area	Area of the island (m^2)
Altitude	Altitude of the island (m)
DistanceToLand	Shortest distance to land (m)
DistanceToLargeIsland	Shortest distance to nearest large island (m)
DepthToCoastMax	Maximum depth to coast (m)
TemperatureMean	Mean annual temperature (°C)
TemperatureMin	Minimum temperature recorded in the coldest month of the year (°C)
TemperatureMax	Maximum temperature recorded in the warmest month of the year (°C)
TemperatureRange	Annual temperature range (°C)
PrecipitationMean	Mean annual precipitation (mm)
PrecipitationMin	Precipitation in the driest month of the year (mm)
PrecipitationMax	Precipitation in the wettest month of the year (mm)
PrecipitationRange	Annual precipitation range (mm)
SolarRadiationMean	Mean annual solar radiation (kJ m^{-2}, day^{-1})
SolarRadiationMin	Solar radiation in the darkest month of the year (kJ m^{-2}, day^{-1})
SolarRadiationMax	Solar radiation in the lightest month of the year (kJ m^{-2}, day^{-1})
SolarRadiationRange	Annual solar radiation range (kJ m-2, day^{-1})
WindSpeedMean	Mean annual wind speed (m s^{-1})
WindSpeedMin	Wind speed in the calmest month of the year (m s^{-1})
WindSpeedMax	Wind speed in the windiest month of the year (m s^{-1})
WindSpeedRange	Annual wind speed range (m s^{-1})

Data from both sources consisted of a series of georeferenced tiff files, which were processed as raster layers and handled with QGIS v.3.6.2 (QGIS Development Team, 2015) to extract the values for each variable at each sampling point. Ecological data on temperature, precipitation, solar radiation and wind speed were acquired from WorldClim database for the period of 1970-2000, and at spatial resolution of 30 seconds (~1 km^2). Data on altitude and area of each sampled islet was extracted from previously published studies of Croatian Islets (Drenovec, 2012; Duplačić Leder et al., 2004). For two mainland populations (SN and ST) the area variable was set to an extremely high value (10,000,000) to reflect their role as mainland populations in comparison with the limited areas of the islands.

Sea batimetry data were downloaded from the EMODnet webpage, and distance to the main land or nearest large island was measured with distance.measureLine tool in QGIS. Profile Tool plugin in QGIS was used to draw the linear characterization of the batimetry from each islet to the mainland in order to obtain the maximum and average depth of its profile. Values of geographic longitude and latitude of sampling sites were used in the analysis to investigate the impact of geographical distance on population's phenotypic and genomic divergence. Area, altitude, distance to land or nearest large island, and depth to coast were considered ecological variables because they largely determine the abiotic and biotic conditions on the islands, and directly affect the probability of predator abundance or invasion, anthropological influence and flora dispersal.

All ecological and geographic variables were standardized before further processing (i.e. subtracted the mean and divided by the standard deviation of the variable across populations) to account for different scales of measurement between distinct variables.

3.3 Genotyping-by-sequencing

3.3.1 GBS library preparation and sequencing

In total, 609 DNA samples (10-47 per population) from 26 wild populations of *P. siculus* and *P. melisellensis*, and 236 additional samples from lizards in the common garden experiment were processed in the laboratory for population genomic analyses. Approximately 15mg of sampled tail tissue was flash frozen in liquid nitrogen to improve mechanical disruption and extraction efficiency, and then minced with scissors. Genomic DNA was extracted with commercial kits (Sigma Aldrich-GenElute Mammalian Genomic DNA Miniprep Kit), using their provided protocol. The quality and quantity of extracted DNA was checked by agarose

gel electrophoresis and spectrophotometric measurement on a Nanodrop (NanoDrop 2000c Thermo Scientific). Extracted DNA was preserved at -20 °C.

GBS sequencing libraries were prepared according to customized protocols from Parchman et al. (2012) and Peterson et al. (2012), which were adapted for pair-end sequencing. 7 µl (150-550 ng) of extracted genomic DNA was first digested by incubation at 37 °C for 8 hours with 3 µl of reaction mix containing 1.15 µl of 10X T4 buffer, 0.25 µl of nuclease free water (nfH_2O), 0.6 µl of 1 M NaCl, 0.6 µl of1 mg/mL BSA, and 0.28 µl of EcoR1 and 0.12 µl MseI restriction endonuclease enzymes (New England BioLabs). Second, custom made EcoR1 adaptors containing 8-10 bp long barcodes that differed by a minimum of 4 bases, and a Y-shaped MseI adaptor (Table 3), were ligated on the digested DNA. In order to get the annealed, double-stranded adaptors, 100 µM stocks of single-stranded oligonucleotides were first mixed with nfH_2O, heated to 95 °C for 5 minutes and slowly cooled down to room temperature. Digestion product was then incubated at 16 °C for 6 hours with 2.4 µl of ligation mix containing 1 µl of each adaptor working stock (EcoR1 final concentration 1 µM and MseI final concentration 10 µM), 0.072 µl of nfH_2O, 0.1 µl of 10X T4 buffer, 0.05 µl of 1M NaCl, 0.05 µl of 1 mg/mL BSA, and 0.1675 µl of 400 U/µl T4 DNA ligase (New England BioLabs). Lastly, the digestion-ligation products were diluted up to 100 µL with 0.1X TE, and 4 µl of diluted product amplified in a 20.15 µl reaction containing 4 µl of 5× Iproof buffer, 9.67 µl of nfH_20, 0.4 µl of 50 mM $MgCl_2$, 0.4 µl of 10 mM dNTP, 0.15 µl of DMSO, 1.33 µl of primer working stock (2.5 µM of each Illumina PCR compatible primer; Table 3), and 0.2 µl of 2 U/µl iProof Polymerase (Bio-Rad). PCR conditions included 98 °C for 30 s, followed by 16 PCR cycles (98 °C for 20 s; 60 °C for 30 s; 72 °C for 40 s) and a final extension at 72 °C for 10 min. The quality of PCR products was checked on agarose gel, after which the samples were pooled together to be sequenced per lane.

The prepared libraries were sent to the BGI sequencing company in Hong-Kong for further processing. BGI provided services of libraries quality control (using Agilent 2100 Bioanalyzer and Real-time Quantitative PCR), gel size selection of DNA fragments from 250 to 450 bp, and 150 bp pair-end GBS sequencing on Illumina HiSeq X Ten platform with 40% PhiX. The company also provided services of initial quality control, de-phixing and demultiplexing of obtained reads. Obtained data were delivered in FASTQ format.

Table 3 Sequences of adaptors and primers used in the library preparations. Barcodes imbedded in EcoR1 adaptor are marked with red X. The asterisks between the first three bases in PCR1 primer mark phosphothiolate modifications.

Oligo name	Sequence 5'	3'
EcoR1_1	AATTGXXXXXXXXXAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT	
EcoR1_2	CTCTTTCCCTACACGACGCTCTTCCGATCTXXXXXXXXXC	
Mse1_1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	
Mse1_2	TAAGATCGGAAGAGCGAGAACAA	
PCR1	A*A*TGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	
PCR2	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC	

3.3.2 Quality control, alignment and variant detection

Raw reads ranged in length from 98 to 150 bp. All raw reads were checked, trimmed of residual adaptor and/or barcode contamination, and standardized to 98 bp for forward reads and 100 bp length for reverse reads using custom made Perl scripts. Reads with uncalled bases were removed, those with average Phred quality score bellow 20 were discarded (default sliding window size of 0.15), and cut sites with one mismatch rescued using *process_radtags* program in Stacks v.2.2 (Catchen et al., 2013; Rochette et al., 2019). Both raw and processed reads quality was checked using FastQC v.0.11.8 (Andrews et al., 2010) and MultiQC v.1.0 (Ewels et al., 2016) software.

Filtered reads were mapped on the reference genome using Bowtie2 software, a short read aligner program that enables the alignment of large sets of short DNA sequence reads to large genomes (Langmead and Salzberg, 2012). The unpublished reference genome, assembled from a *P. siculus* female individual from Pod Mrčaru, was provided by Rasmus Nielsen research group from Berkeley University in the USA (Supplementary material, Table S2). Reference genome was first indexed, and reads then mapped to it using the default Bowtie2 v.2.3.4.1 settings. Aligned reads were transformed from SAM to BAM format using Samtools v.1.9 (Li et al., 2009).

Mapped reads were run through Stacks v.2.2 *ref_map* pipeline (Catchen et al., 2013; Rochette et al., 2019) in order to detect variant sites. First, loci were assembled according to the alignment positions provided for each read, and SNPs called across all samples with *gstacks* program. Maximum-likelihood ‘marukilow’ model was applied to account for statistical

uncertainties associated with sequencing errors during variant (alpha threshold = 0.01) and genotype (alpha threshold = 0.05) calling. Default settings were applied for all other *gstacks* program parameters, apart for the minimum mapping quality to consider a read, which was set to more conservative value of 20. Second, the SNPs were filtered according to their quality and position, and the population-level summary statistics were generated using Stacks *populations* program. Parameters for *populations* program included: restricting SNP calling to one SNP per locus; setting the minimum percentage of individuals across all populations required to process a locus to 70% and minimum percentage of individuals in each population required to process a locus for that population to 60%; minimum number of populations a locus must be present was set to 100%; minimum minor allele frequency required to process a nucleotide site to 0.05; and maximum observed heterozygosity to process a nucleotide site to 0.6. Variant call format (VCF) file produced by *populations* program was checked using custom made Perl scripts in order to identify samples containing more than 25% of missing values. Stacks *ref_map* pipeline was then repeated using the same parameters specified above, but excluding 13 samples that didn't pass the missing values threshold.

Variant sites with mean coverage depth lower than 4X and larger than 20X were filtered out using VCFtools v0.1.13 (Danecek et al., 2011). Additional data filtering to reduce the number of missing values was done using custom made Perl scripts. Loci with more than 25% of missing values were deleted and population's most frequent known genotype imputed for any remaining missing values (if two or more genotypes present in the population had the same highest frequency, one was assigned at random).

The final dataset contained 19550 SNPs genotyped across 832 individuals from 26 wild populations, as well as the two additional generations of Pod Kopište and Pod Mrčaru and F1 juveniles used in the crossing experiment. In order to investigate genomic patterns of wild populations in depth, 4 different subsets were further produced by extracting specific groups of populations and excluding any resulting monomorphic loci (Table 4). Final number of genotyped samples per population is provided in the Supplementary material, Table S3.

PGDSpider v.2.1.1.5 (Lischer and Excoffier, 2012) was used to convert genotype datasets from VCF format to other program-specific input files. For LFMM and RDA analyses, genomic datasets were further transformed from Genlight format to individual allele count (0/1/2) format using functions available in R packages *adegenet* and *dartR* (Gruber et al., 2018; Jombart, 2008). To convert genomic files from Bayscan/Geste to Baypass format, a

Python script *geste2baypass* was used (available at: https://github.com/CoBiG2/RAD_Tools /blob/master/geste2baypass.py).

Table 4 Subset datasets created for population genomic analyses.

Dataset	Populations	Samples	SNPs
all wild *Podarcis* populations	26	600	19550
wild *P. siculus* populations	14	362	12056
wild *P. melisellensis* populations	12	238	13538
2016 wild PM and PK populations	2	87	2421

3.4 Genomic patterns in wild populations

3.4.1 Genomic diversity and population differentiation estimates

Genomic diversity within the populations was estimated using statistical indices of observed and expected heterozygosity (Ho and He respectively), allelic richness (Ar) and inbreeding coefficient (F_{IS}). Diversity indices were obtained for all 26 wild *Podarcis* populations (Table 4) using *diveRsity* v.1.9.90, *hierfstat* v.0.5.7 and *adegenet* v.2.1.3 packages in R 4.0.0 (Goudet, 2005; Jombart, 2008; Keenan et al., 2013; R Core Team, 2017). Significance of Hardy-Weinberg equilibrium test was calculated using 1000 iterations, and 99 bootstrap replicates were used to calculate confidence intervals for F_{IS} and Ar. Calculation of pairwise indices of genetic differentiation (F_{ST}) between populations was performed using *StAMPP* package v. 1.6.1 (Pembleton et al., 2013), set with 99,999 bootstrap replicates across loci to calculate p-values and assess the confidence of the estimate. Principal component analysis (PCA) of allelic frequencies across all four genomic datasets (Table 4) was conducted using *adegenet* package v.2.1.3 (Jombart, 2008). In order to test for isolation by distance, Mantel test between pairwise genomic (F_{ST}) and geographic distances was performed as implemented in *ape* v.5.4.1 and *vegan* v. 2.5.6 packages, using 999 replicates (Oksanen et al., 2019; Paradis and Schliep, 2019).

Individual ancestry and genomic structuring in native populations was further examined using *fastStructure* v.1.0 software (Raj et al., 2014). This software distributes the samples in the specified number of clusters until it finds the combination of allelic frequencies that minimizes the deviation from Hardy-Weinberg equilibrium inside each cluster. A Bayesian

framework is used to iterate the same analysis multiple times and infer the most probable number of genetically different clusters. To compare between inter and intra-specific patterns of population structuring, analyses were performed on three different datasets, composed of either all *Podarcis* populations, or only *P. siculus* and *P. melisellensis* individuals (Table 4). Since *fastStructure* does not explicitly account for linked markers or markers out of Hardy-Weinberg equilibrium (HWE), datasets were first filtered for linkage disequilibrium (LD) using Plink v.1.9 (Purcell et al., 2007) software. Correlations between genotype allele counts were examined using a window size of 50, step size of 5, and r^2 threshold of 0.5, and pruned subsets of markers in linkage equilibrium with each other were produced (4374 SNPs from wild *Podarcis* dataset, 8295 SNPs from wild *P. siculus* dataset, and 9066 from wild *P. melisellensis* dataset). Next, R packages *pegas* v.0.13 (Paradis, 2010) and *DartR* v. 1.1.11 (Gruber et al., 2018) were used to test for loci that were significantly out of HWE ($p<0.05$; 99 replicates) in more than 60% of populations. Since all LD filtered loci passed the HWE test, none of them were excluded from subsequent *fastStructure* analyses. Because uneven sampling can lead to biased inferences on hierarchical structure (Puechmaille, 2016), genomic datasets were further randomly subsampled to a maximum of 19 samples per population using custom-made Perl script. To choose the appropriate number of clusters (K) that best explain the genetic structure, *fastStructure* was run independently for K ranging from 1 to a number of populations sampled. Default inference admixture model was used in all *fastStructure* analysis. Ideal number of clusters was chosen based on model complexity that maximizes marginal likelihood, or the number of components used to explain structure in the data. Software *distruct* v.1.1 (Rosenberg, 2004) was used to graphically display individual membership coefficients to each of the cluster.

Analysis of molecular variance (AMOVA) was implemented to estimate the amount of genomic variance among and within species, populations, and genomic clusters identified in the *fastStructure* analysis. Three separate hierarchical AMOVA analyses were run using Arlequin v.3.5 (Excoffier and Lischer, 2010) with 1,000 permutations to assess statistical significance of fixation indices. First, genomic variance was partitioned between species and among genomic clusters identified within each species, using dataset composed of all 26 *Podarcis* populations (Table 4). Next, two separate analyses of *P. siculus* and *P. melisellensis* datasets (Table 4) were conducted to estimate variance components among genomic clusters and corresponding populations for each species.

3.4.2 Identification of candidate loci for selection

Four different analytical approaches were employed to identify putative loci under selection in Pod Mrčaru and Pod Kopište *P. siculus* populations, i.e. the loci that would be indicative of adaptive genomic divergence. All analyses were conducted on genomic dataset consisting of only Pod Mrčaru and Pod Kopište *P. siculus* populations (Table 4).

First, a Bayesian F_{ST}-based approach to estimate the posterior probability of each locus to be under selection was employed using BayeScan software v.2.1 (Foll and Gaggiotti, 2008). This method uses logistic regression to separate F_{ST} coefficients into population-specific (neutral or demographic variation) and locus-specific component (adaptive variation), with positive values of locus-specific component suggesting diversifying, and negative values balancing or purifying selection. BayeScan was run using default MCMC settings (20 pilot runs of 5,000 iterations, followed by 100,000 iterations with 50,000 burn-in and a thinning interval of 10) and prior odds for the neutral model (pr_odds 10). Outputs were processed in R using provided *plot_R.r* script. Statistical significance was assessed based on obtained loci-specific q-value – a false discovery rate (FDR) analogue of the p-value. Loci were considered candidates if they showed q-value lower than 0.05 threshold.

Next, the dataset was tested using a genome scan method implemented in Arlequin v.3.5 (Excoffier and Lischer, 2010), which detects loci putatively under selection by analysing joint distribution of F_{ST} and heterozygosity under simulated neutrality. Arlequin analysis was performed using non-hierarchical finite island model, testing 10,000 simulations with 100 demes. Loci with positive F_{ST} values (denoting directional selection) and p-value lower than 0.05 were selected as putative outliers under selection.

Another genome scan for selection was implemented using *PCAdapt* package v.4.3.3 (Luu et al., 2017) in R. *PCAdapt* method uses a multivariate PCA approach which identifies outliers in respect to how they relate to population structure, without assuming membership of samples to populations or groups. More simply, *PCAdapt* detects candidate loci by looking at correlations between SNPs and a set of principal components (K). The optimal K value was chosen by running analysis with K = 1-10, and assessing score plots for explained levels of population structure. Only the first principal component (K = 1) was retained as it was the only one contributing to differentiation between two islands. *PCAdapt* significant loci were considered those with p-value lower than 0.05. To control for false positives, only loci

identified as significant in two out of three genome scan analyses were considered as candidate loci putatively under selection in Pod Mrčaru and Pod Kopište populations.

Finally, the candidate loci obtained by three genome scans methods were checked to see if they also contribute to the genomic divergence between the two populations. To that end, PC1 loadings were extracted from results of the PCA analysis of Pod Kopište and Pod Mrčaru dataset preformed using *adegenet* v.2.1.3 (Jombart, 2008) package in R (see section 3.4.1 Genomic diversity and population differentiation estimates). In order to detect loci showing highest variation in allele frequencies driving the separation of populations along first PCA axis an arbitrary threshold of top 15% was applied to obtained loading values. The contribution of those loci to genomic divergence between focal populations is irrespective of the relative influence of selection and demographic effects.

3.4.3 Phenotypic differentiation and genotype-phenotype associations

Phenotypic differentiation among *P. siculus* and *P. melisellensis* populations sampled in the wild was first explored using a PCA analysis of 13 quantitative traits of the head and body obtained for each individual (Table 1; excluding snout-vent length). Variation in phenotype was analysed in respect to species, sex and sampling sites, using the principal component analysis from R package *stats* (R Core Team, 2017).

Next, the degree of phenotypic differentiation between Pod Mrčaru and Pod Kopište *P. siculus* populations was assessed using the P_{ST} index for each investigated trait (Table 1). P_{ST} approximates Q_{ST} (standardized measure of genetic differentiation of quantitative traits among populations; see Spitze (1993)), but does not require detailed estimation of additive genetic variance component underlying traits of interest, making it useful in evolutionary ecology studies of wild populations. Similarly to Q_{ST}, P_{ST} statistics can be compared with F_{ST}, leading to three possible outcomes: 1) $P_{ST} > F_{ST}$ suggests higher divergence in quantitative traits than in neutral markers, indicative of directional selection; 2) $P_{ST} < F_{ST}$ indicates stabilising influence of natural selection, with same phenotypes being favoured in different populations; 3) $P_{ST} = F_{ST}$ indicates no departure from neutral expectations, where drift and selection effect on population differentiation cannot be separated. P_{ST} values were calculated separately for each sex and phenotypic trait using *Pstat* R package v.1.2 (Silva and Silva, 2018). Because values of h^2 (narrow-sense heritability) and c (proportion of the total variance due to additive genetic effects across populations) parameters could not be readily estimated for all traits using our experimental design, and to ensure that the results were not

affected by assumptions regarding modelled c/h^2 ratio, several P_{ST} calculations were performed using different c/h^2 parameter values (0.25, 0.5, 0.75 and 1). Confidence intervals were estimated after 1000 bootstrap iterations. Obtained P_{ST} values were compared with genomic differentiation index computed previously (population-pairwise F_{ST} values; see section 3.4.1 Genomic diversity and population differentiation estimates).

Genotype-phenotype associations (GPA) were explored using latent factor mixed models (LFMM) analysis on genomic dataset containing only Pod Mrčaru and Pod Kopište populations (Table 4), and 14 phenotypic traits obtained for each sampled individual (Table 1). LFMM approach is similar to mixed model regression often used in GWAS, which test associations between a multidimensional set of response variables (genotypes) and a set of variables of interest (phenotypic traits or environmental exposure). However, unlike standard mixed models that employ kinship matrix or principal components, LFMM corrects for confounding effects due to population structure and other hidden causes by including random unobserved variables K (called latent factors). Although phenotype is usually considered a response and genotype an explanatory variable in biological sense, LFMM corrects for the confounding effects of latent factors by modelling them together with the response variables. Consequently, LFMM genotype-phenotype association test used in this study was performed with genotypes modelled as a response and phenotype as explanatory variable. LFMM analysis was run using *ridge* analytical method from R package *lfmm* (Caye et al., 2019), fitted with two latent factors (K = 2) identified by PCA analysis of genomic data from Pod Mrčaru and Pod Kopište populations (see section 3.4.1 Genomic diversity and population differentiation estimates). Obtained z-scores were further recalibrated with modified genomic inflation factors (GIFs) following the procedure described in Frichot and François (2015), in order to obtain a uniform p-value distribution which is expected under the null-hypothesis. Loci showing significant association with analysed phenotypic variables were determined by Benjamini-Hochberg procedure on adjusted p-values with false discovery rate (FDR) = 0.05. To see if markers associated with phenotype also showed signatures of directional divergence in Pod Mrčaru and Pod Kopište populations, GPA significant loci were compared with candidate SNPs detected in genome scans for selection (see section 3.4.2 Identification of candidate loci for selection). Additional LFMM association tests were also performed on genomic datasets containing 14 wild *P. siculus* or 12 wild *P. melisellensis* populations (Table 4).

3.4.4 Genotype-environment associations

Genotype-environment associations (GEA) were assessed using wild *P. sicula* and *P. melisellensis* genomic datasets (Table 4) and 23 environmental variables obtained for each sampling site (Table 2, Supplementary material, Tables S4 and S5). Genotypic variance was first partitioned between spatial (neutral) and ecological (adaptive) component using a multivariate redundancy analysis (RDA) approach. RDA is a constrained linear ordination method in which multiple regressions are fitted between response (individual genotype) and explanatory variables (environment). PCA is then preformed on the fitted values to extract the RDA axes, which represent linear combinations of explanatory variables that best explain the variation in the response matrix. To account for correlation among explanatory factors, PCA was performed on standardised ecological variables (Table 2) and scores from the principal components that explained more than 10% variance were extracted to be used in RDA analysis. Geographic variation among populations was modelled with distance-based Moran's eigenvector maps (dbMEMs), a spatial eigenfunction method that decomposes physical distances into a new set of independent variables appropriate for subsequent RDA analyses. Raw geographic latitude and longitude values were transformed to Cartesian coordinates, and dbMEMs variables obtained through a Euclidian distance matrix using *SoDA* v.1.0.6 and *adespatial* v.0.3.8 packages in R (Chambers, 2013; Dray et al., 2020). Only positive Moran's eigenvectors were retained to be used in RDA analysis. Three different types of RDA analyses were performed using functions available in R package *vegan* v.2.5.6 (Oksanen et al., 2019): full RDA analysis with both ecological and geographical data as explanatory variables, partial RDA analysis with ecology as explanatory and dbMEMs as conditioning variables, and partial RDA with dbMEMs as explanatory and ecological data as conditioning variables. Variance partitioning between ecological and/or spatial distance components was based on inertia and adjusted R^2 values from the respective RDA analyses. Significance of the model, RDA canonical axes, and marginal effects of explanatory variables were tested using ANOVA after 999 permutations. RDA analysis was additionally performed on *P. siculus* dataset using only putatively adaptive loci identified in two focal populations (see section 3.4.2 Identification of candidate loci for selection).

Secondly, three different Bayesian approaches were employed to explore univariate relationships between each loci and environmental variable separately. The datasets were first tested using a well-established Bayesian algorithm implemented in Bayenv2 software v.2.0 (Günther and Coop, 2013). This approach allowed detecting the correlation between changes

in allele frequency distributions and differences in environmental factors among sites, while simultaneously controlling for the effect of population structure by incorporation of covariance matrix (which is expected to be closely related to the matrix of population pairwise F_{ST}). Population covariance matrices were first estimated using all available genomic variants and 100,000 MCMC iterations over 5 replicate runs of Bayenv2 program. Last matrices outputted across independent runs were averaged and mean covariance matrix used in all subsequent analyses. Covariance matrix was transformed to correlation matrix and compared with previously obtained population-pairwise F_{ST} values (see section 3.4.1 Genomic diversity and population differentiation estimates) using Mantel test available in *ecodist* v.2.0.5 package in R (Goslee and Urban, 2007). Bayenv2 GEA analysis was then performed using the mean estimated covariance matrix. Analysis was again repeated in 5 replicates using 100,000 iterations to reduce variability produced by stochastic error among different MCMC runs. Significant SNPs were considered those with mean Bayes factor (BF) > 5 and falling within top 5% of mean Spearman correlation coefficient values across all replicate runs.

Genotype-environment associations were further assessed using Baypass v.2.1, an extension of Bayenv2 method developed by Gautier (2015), which refines covariance matrix calculation through the use of a hierarchical Bayesian model and implements calibration procedure for XtX statistics to identify SNPs under selection. Baypass was run in several successive steps. First, the core model was explored in order to obtain the population covariance matrix and estimate XtX statistics for outlier loci detection. Next, pseudo-observed datasets (PODs) with 10,000 SNPs were simulated using *simulate.baypass* function available in *baypass_utils* R source package, and covariance matrix and beta parameters calculated under the core model in previous step. PODs were then analysed with the core model in the same way as the real data, and 0.01 and 0.99 quantiles of the XtX distribution from POD analyses calculated in order to provide a cut-off value to discriminate between neutral and outlier loci for XtX statistics obtained on real datasets. Loci with mean XtX values lower or higher than the obtained 1% and 99% threshold were considered outliers under balancing or directional selection, respectively. The core model was then run once again using only neutral loci (outlier loci under directional or balancing selection excluded from dataset). Lastly, Baypass was run under auxiliary model (AUX), with the neutral covariance matrix obtained in previous step and 23 environmental variables to test for GEA. SNPs considered strongly associated with the environment were those with BF values > 15

dB (deciban units). All Baypass analyses were performed 5 independent times with different seeds. Convergence of algorithm was confirmed by assessing correlations of the estimated parameters among runs, and mean values across replicate runs were taken as the final result. Similarity among covariance matrices obtained on empirical and simulated datasets was verified using Förstner and Moonen distance (FMD < 1) statistics from R function *fmd.dist* included in BayPass. Similarity between Baypass covariance matrices and population-pairwise F_{ST} values was explored using the same method as described for Bayenv2 analysis. MCMC parameters across all Baypass analyses included 30 pilot runs with 5,000 iterations each, followed by 50,000 steps of burn-in, and 100,000 post burn-in iterations with a thinning interval of 25.

Lastly, GEA analysis were conducted using BayeScEnv v1.1 (de Villemereuil and Gaggiotti, 2015), a Bayesian ecological association method based on BayeScan software. This method extends the previously described BayeScan approach from Foll and Gaggiotti (2008) by introducing an additional model that includes information about locus-specific effect of local adaptation caused by analysed environmental variable. BayeScEnv analysis was run using default values for prior probabilities (prior jump probability of 0.1 and prior preference for alpha model of 0.5) and MCMC chain (20 pilot runs with 5,000 iterations, followed by 100,000 iterations with 50,000 burn-in and a thinning interval of 10, resulting in 5,000 outputted iterations). Model performance (autocorrelation, convergence, and effective sample sizes) was checked using functions available in *coda* v. 0.19.3 package in R (Plummer et al., 2005). Acceptance rates were analysed directly from the output of BayeScEnv analysis. Loci with q-value < 0.05 were considered associated with analysed environmental variables.

Results from the three Bayesian GEA analyses were compared with candidate loci detected in genome scans for selection in Pod Mrčaru and Pod Kopište dataset (see section 3.4.2 Identification of candidate loci for selection), in order to identify environmentally associated genomic markers important for adaptive differentiation between these two focal populations. Furthermore, to asses if the number of loci found in common between candidate loci for selection in the two focal populations and Baypass core model analysis on all *P. siculus* was greater than expected by chance, 100000 sets of random loci (containing the same number of loci that were identified as putatively under selection in two focal populations or in all *P. siculus*) were generated from the total list of loci in R. Each generated random set was then overlapped with significant loci from the other analyses and the quantiles of obtained overlap

distributions (95%, 99%, 99.9%, and 99.99%) compared to the real number of loci found in common across the two analyses.

4. RESULTS

4.1 Crossing experiment in the common garden

In 2017, only 26 F1 offspring that lived past 18 months of age were obtained from crossings set between and within Pod Kopište (PK) and Pod Mrčaru (PM) populations. However, change in vermiculite used for egg incubation and preference for established pairs where male and female spent over a year together in a terrarium resulted in greater yield in 2018. In total, 79 F1 full-sib offspring from 62 F0 individuals were raised to sub-adulthood (18 months of age) in the 2017-2019 crossing experiment (Table 5).

Table 5 Families and sample sizes of F1 offspring who reached sub-adulthood within experiment for each cross type (KK = PK♂-PK♀; KM = PK♂-PM♀ = MK: PM♂-PK♀; MM = PM♂-PM♀) in respect to sex (♀/♂) or experimental year (2017/2018).

Cross type	Families	F1	F1♀	F1♂	F12017	F12018
KK	8	22	9	13	8	14
KM	6	15	6	9	7	8
MK	9	24	17	7	5	19
MM	8	18	9	9	6	12
total	31	79	41	38	26	53

4.1.1 Phenotypic variability among experimental individuals

Repeatability of the entire phenotypisation procedure consisting of obtaining photographs and extracting phenotypic measures using geometric morphometry based on landmark data was sufficient across all analysed traits. Repeatability was estimated as 0.91 for head height, head length, lower jaw length and lower jaw outlever, 0.85 for snout length, 0.89 for head width, 0.77 for length to hip, and 0.97 for tail length (Supplementary material, Table S6).

Analysis of phenotypic variability between adult Pod Mrčaru and Pod Kopište individuals that served as F0 generation in the crossing experiment confirmed differentiation in body and head size between these two populations (Figure 6). Both female and male individuals from Pod Mrčaru had larger bodies and higher heads, with length to hip (LtHip) and head height (HHght) measures significantly greater than those recorded in Pod Kopište lizards ($p < 0.01$ and < 0.05, respectively). Male individuals additionally showed significant differentiation (p

< 0.05) in head length (HLgth), lower jaw length (LwJaL) and lower jaw outlever (LwJaO). No significant difference was found for head width (HWdth), snout length (SnLgh), bite force (BiteF) or tail length (TailL) measures in either sex. Nonetheless, the general pattern of Pod Mrčaru *P. siculus* individuals having larger bodies and heads than those from Pod Kopište can be observed across almost all analysed traits (Figure 6).

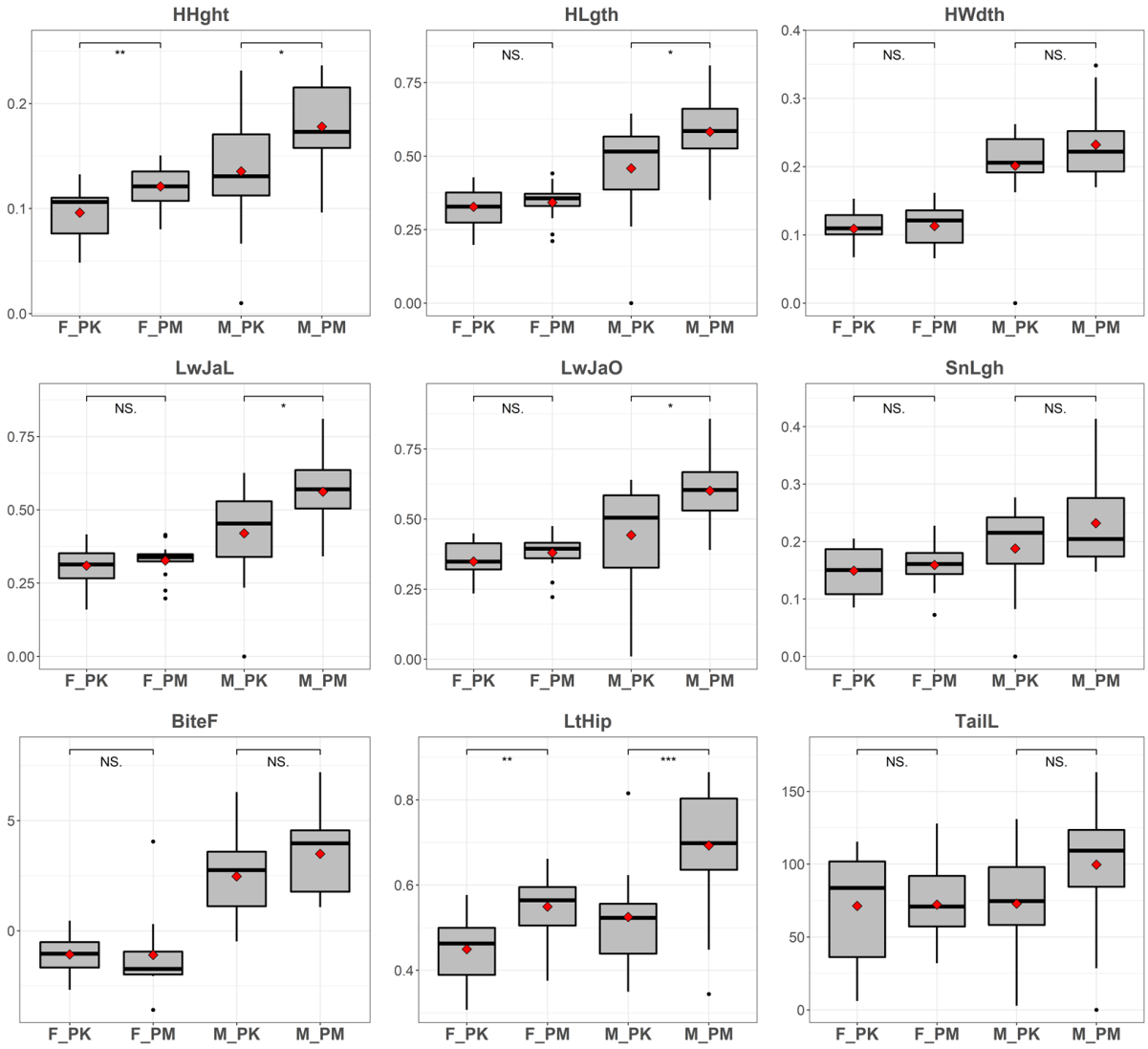


Figure 6 Boxplots illustrating phenotypic trait variability in female (F) and male (M) individuals from Pod Mrčaru (PM) and Pod Kopište (PK). Red rhombus indicates group mean, bold line stands for median, the box represents quartiles and whiskers stand for minimum and maximum recorded values. Pairwise t-test significance is indicated above boxplots (***=0.001, **=0.01, *=0.05, NS.=not significant). Phenotypic trait abbreviations are defined in the text.

PCA analysis of F0 individuals from the common garden experiment demonstrated that most variation in adult phenotype is due to the pronounced sexual dimorphism among *P. siculus* lizards. First two principal components explained 93.58% of the total phenotypic variation, with first principal component accounting for 87.3% variance and second component for 6.28% of phenotypic variation. PCA scatter plot showed clear separation of male and female individuals along the PC1 axis (Figure 7A), and GLM on PC1 scores underlined small, but significant separation according to analysed groups ($p < 0.05$). PC1 correlated with all head size traits, while head width (HWdth) and snout length (SnLgh) contributed the most to the separation of individuals along PC2 axis **(**Figure 7B**)**. PCA analysis of F0 individuals conducted separately for each sex did not show any significant separation between individuals from Pod Mrčaru and Pod Kopište.

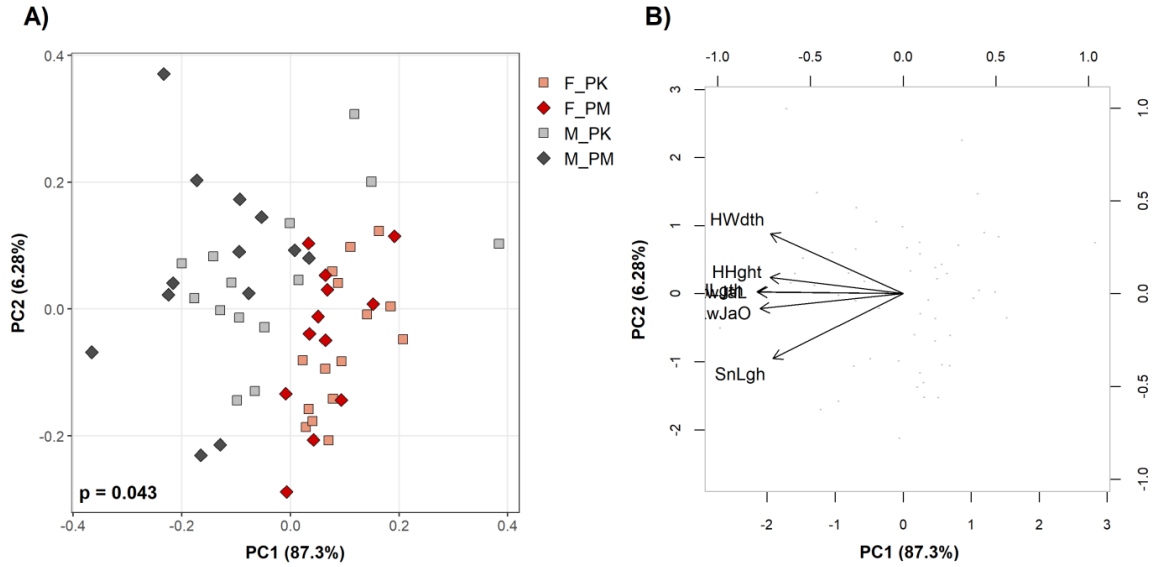


Figure 7 A) Scatter plot and **B)** biplot from PCA analysis of head size traits in female (F) and male (M) Pod Kopište (PK) and Pod Mrčaru (PM) F0 individuals. GLM significance is denoted with p-value in the right left corner of the scatter plot. Phenotypic trait abbreviations are defined in the text.

First two principal components from PCA analysis of female F1 offspring explained 82.83% of the total variance, with first one accounting for 68.75%, and the second for 14.08% of total phenotypic variation (Figure 8A). PCA plot showed some separation between KK (PK♀-PK♀) and MM (PM♂-PM♀) crosses along PC1 axis, and GLM on PC1 scores indicated that phenotype differs significantly among 4 cross types. PC1 further correlated with all head size traits except head width (HWdth) which contributed most to PC2 separation **(**Figure 9A**)**.

In PCA analysis of male F1 offspring first principal component explained 78.46% and second 13.19% of phenotypic variation, accounting for 91.61% of the total phenotypic variance (Figure 8B). Similar to PCA results from female offspring, PC1 was correlated with almost all traits besides HWdth, which showed highest loading on PC2 (Figure 9B). However, no significant separation of groups along PC1 axes was detected among male offspring.

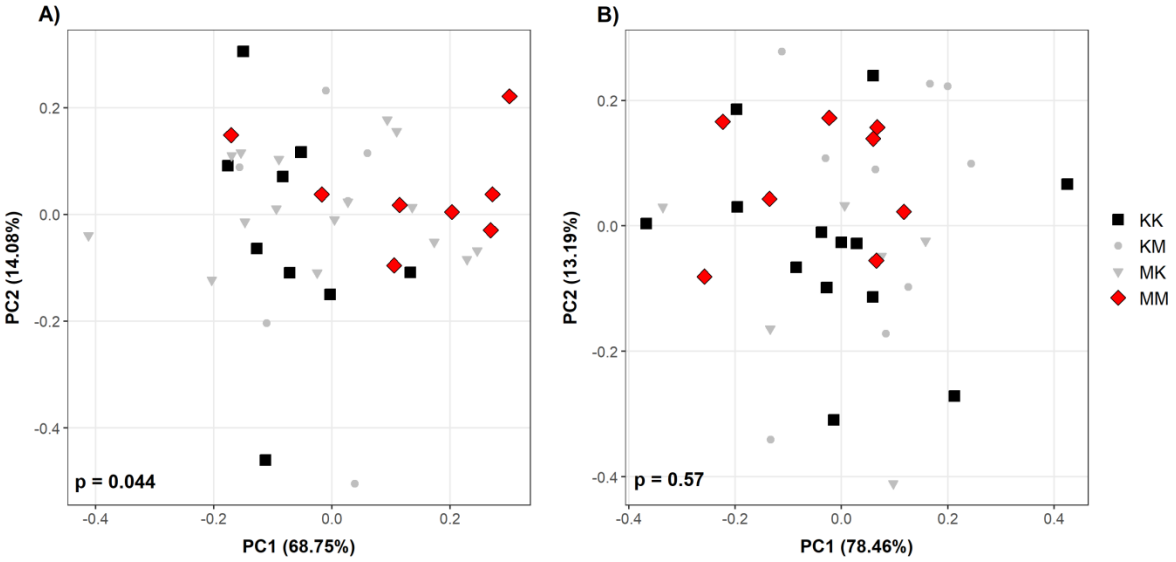


Figure 8 PCA analysis of head size traits in **A)** female and **B)** male F1 offspring, analysed per cross type (KK = PK♂-PK♀; KM = PK♂-PM♀; MK = PM♂-PK♀; MM = PM♂-PM♀). GLM significance is denoted with p-value in the right left corner.

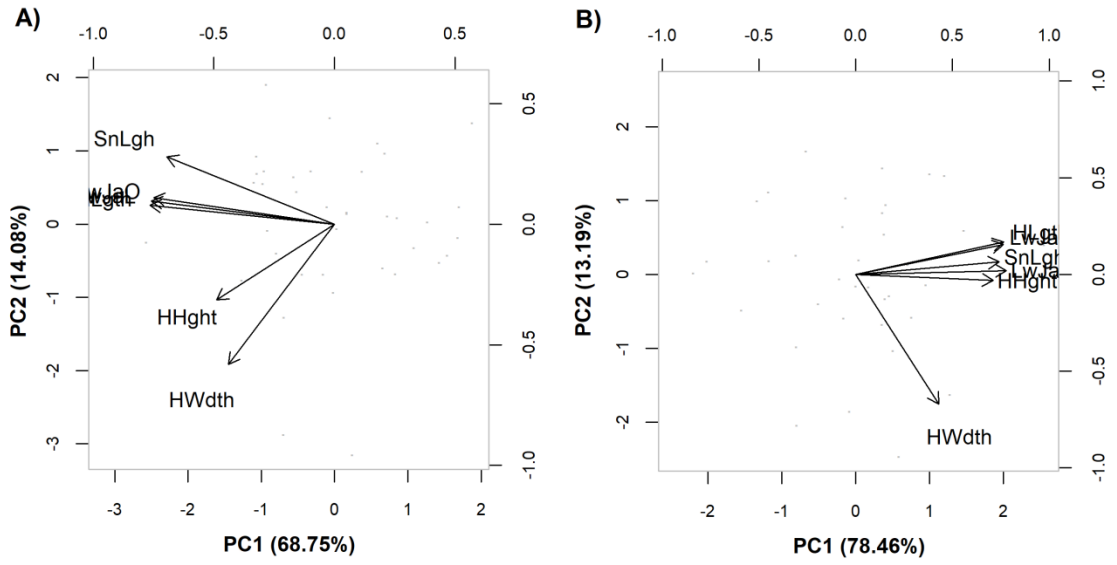


Figure 9 PCA biplot head size traits in **A)** female and **B)** male F1 offspring. Phenotypic trait abbreviations are defined in the text.

Analysis of variance on female F1 offspring showed significant ($p < 0.05$) differentiation in phenotypic measures of head length (HLgth) and lower jaw outlever (LwJaO) between different cross types (Figure 10). However, Tukey's test found only head length (HLgth) measure to be significantly different between KK (PK♂-PK♀) and MM (PM♂-PM♀) crosses. Other traits did not show significant difference among female offspring.

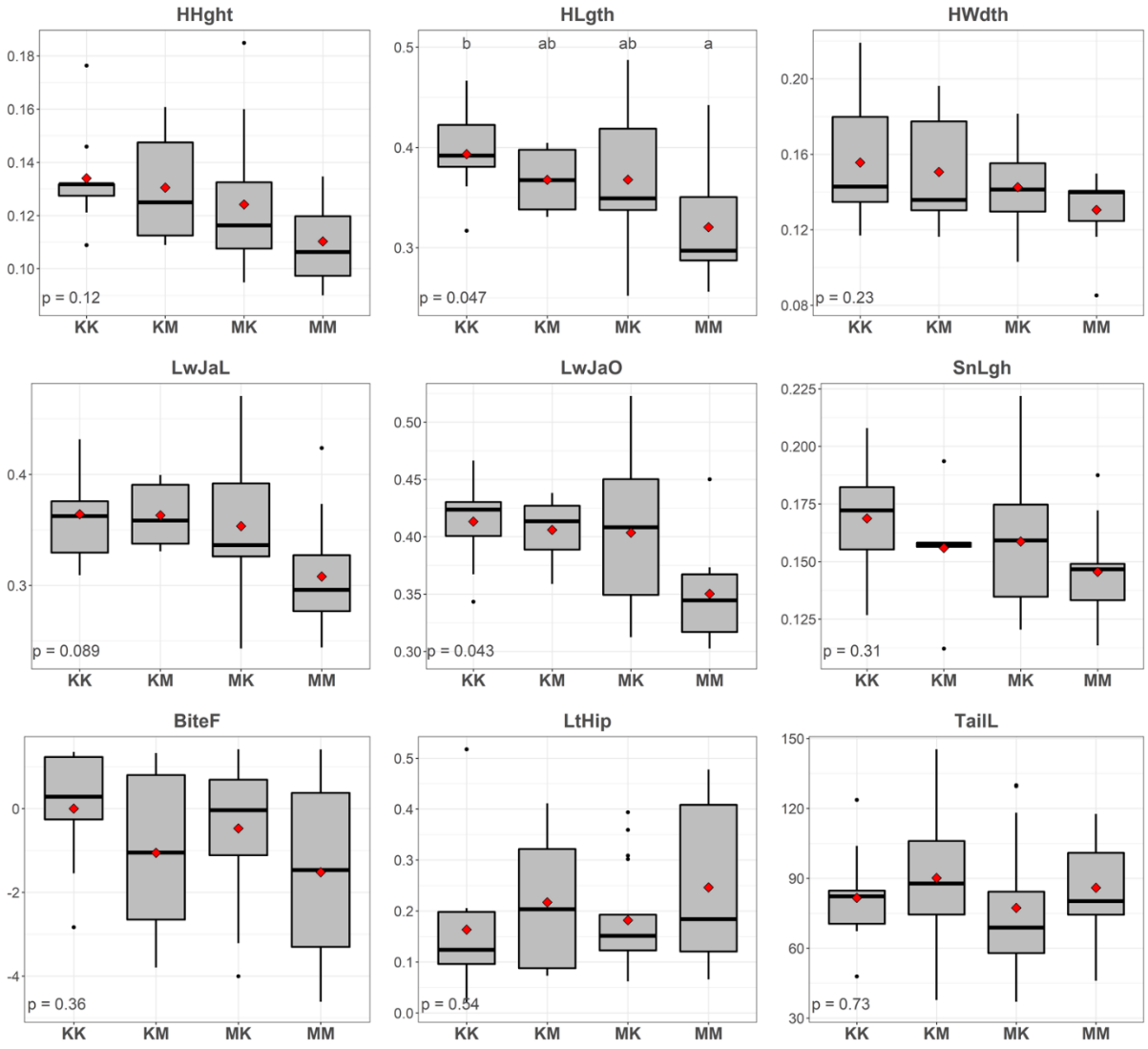


Figure 10 Boxplots illustrating phenotypic trait variability in female offspring in each cross type (KK = PK♂-PK♀; KM = PK♂-PM♀; MK = PM♂-PK♀; MM = PM♂-PM♀). Red rhombus indicates group mean, bold line stands for median, the box represents quartiles and whiskers stand for minimum and maximum recorded values. ANOVA significance is denoted with p-value in the left bottom corner. Different letters above boxplots indicate between-group differences as indicated by Tukey's honest significant difference test. Phenotypic trait abbreviations are defined in the text.

No discernible trend or significant difference was shown in analyses of phenotypic variability among groups of male F1 offspring (Figure 11).

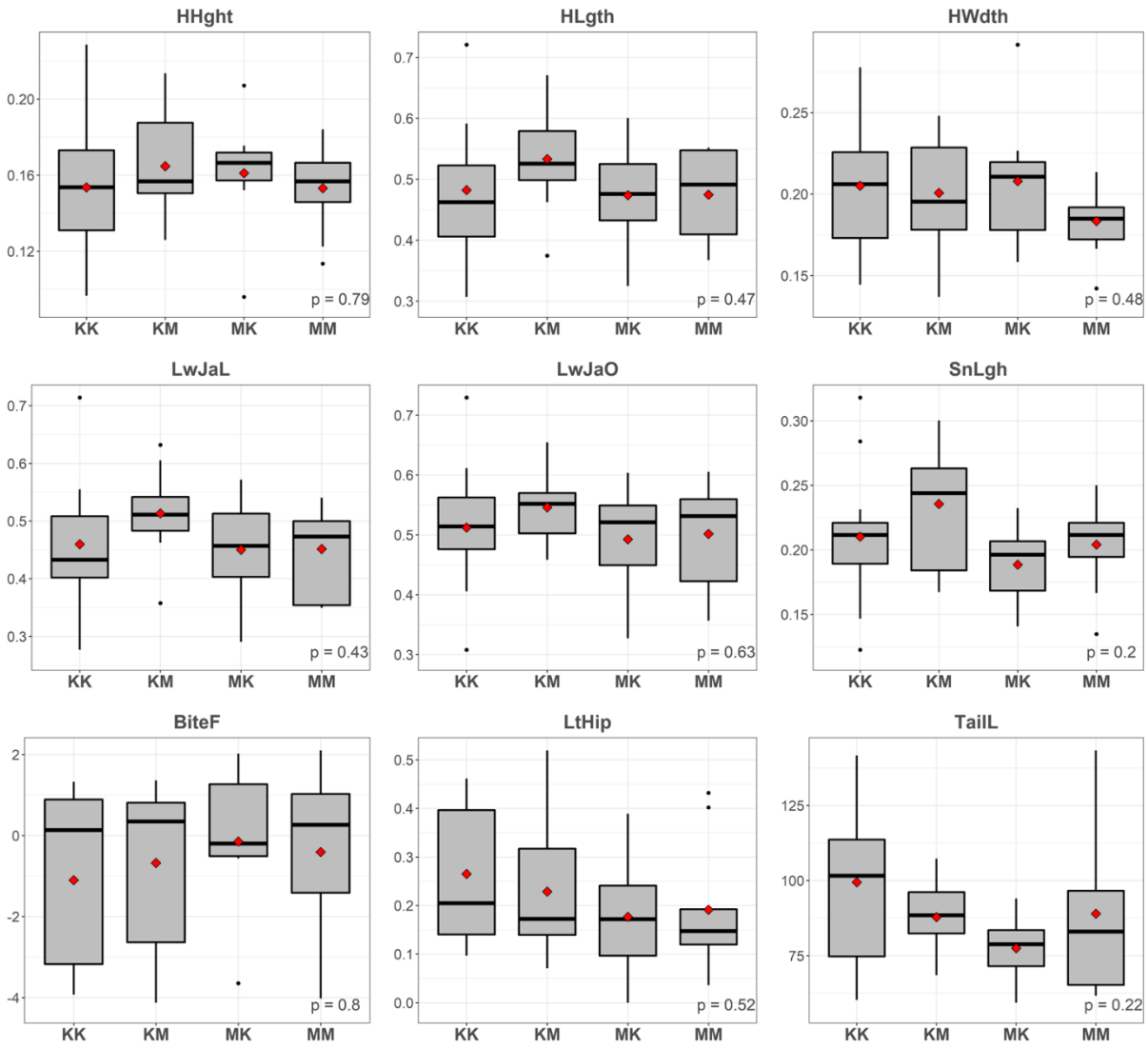


Figure 11 Boxplots illustrating phenotypic trait variability in male offspring in each cross type (KK = PK♂-PK♀; KM = PK♂-PM♀; MK = PM♂-PK♀; MM = PM♂-PM♀). Red rhombus indicates group mean, bold line stands for median, the box represents quartiles and whiskers stand for minimum and maximum recorded values. ANOVA significance is denoted with p-value in the right bottom corner. Phenotypic trait abbreviations are defined in the text.

4.1.2 Heritability estimation

The basic analyses of additive genetic variance influencing variability in divergent phenotypic traits under simple models showed inconsistent results across different analyses (Figure 12). Parent-offspring regression and full-sib ANOVA approaches did not perform well, but several significant results were obtained using other analyses. In particular ASSOC, WOMBAT and MCMCglmm showed high heritability estimates for head width (HWdth; mean value of $h^2 = 0.57$) and head height (HHght; mean value of $h^2 = 0.54$).

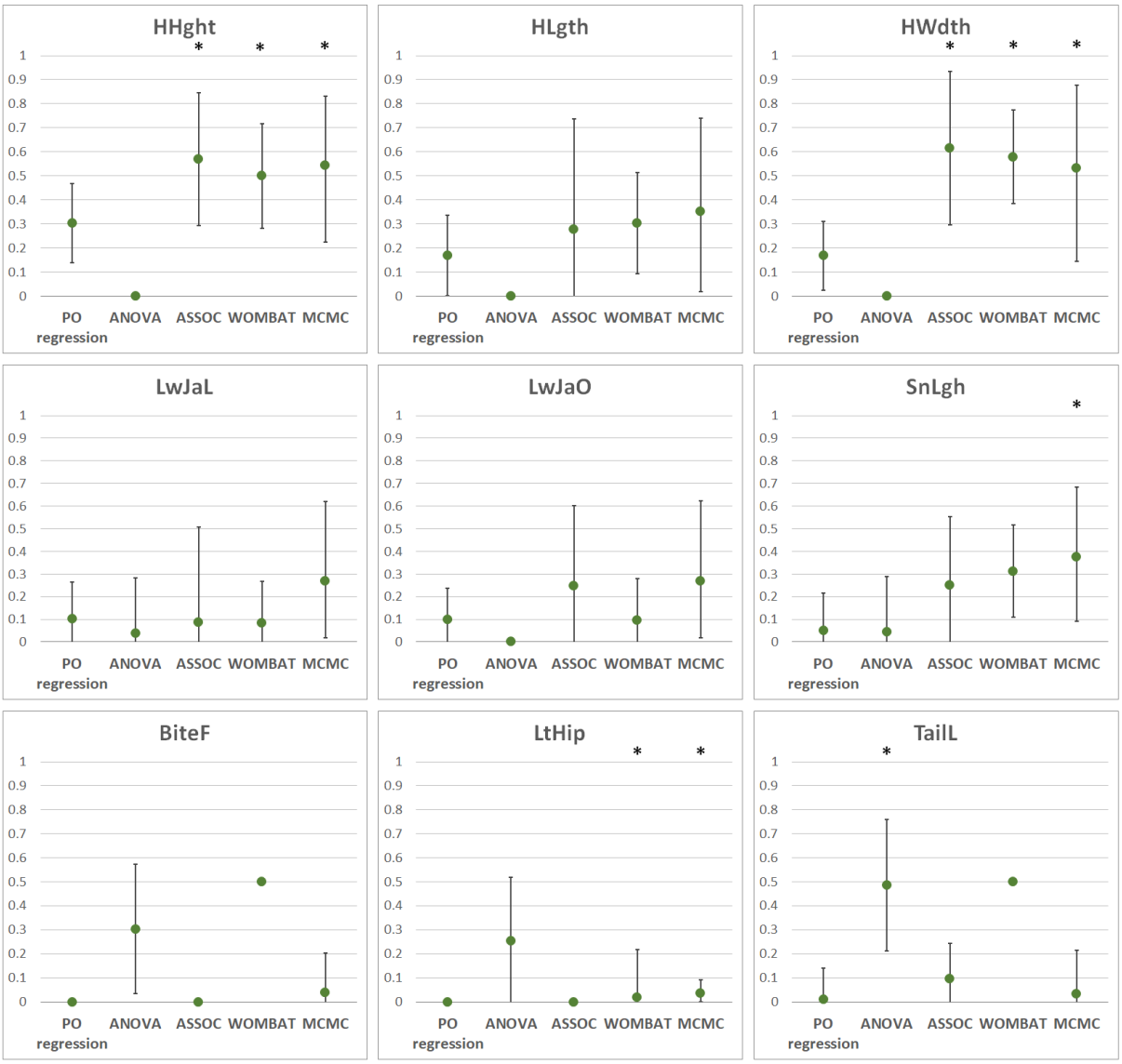


Figure 12 Heritability and associated standard errors (i.e. 95% credible intervals in Bayesian MCMCglmm analysis) estimated under the simple model. Asterisks mark analyses that were significant with $p<0.05$ (PO regression, ANOVA, ASSOC) and models with small autocorrelation which converged successfully (WOMBAT, MCMCglmm). Negative values are expressed as zero. Phenotypic trait abbreviations are defined in the text.

Significant heritability values (h^2 = 0.37) were also recorded for snout length (SnLgh) in MCMCglmm analysis, and extremely low but significant heritability estimates were obtained for length to hip (LtHip) in WOMBAT and MCMCglmm models (mean value of 0.03). Estimates for other traits were not significant and/or did not show good model support. Curiously, while PO regression showed lower, but still positive heritability estimates (> 0.1) for head size, ANOVA analysis on full-sibs gave negative values for most of the same traits. On the other hand, when looking at bite force (BiteF), length to hip (LtHip) or tail length (TailL) – traits for which other analysis showed extremely low or inconclusive results (i.e. low model support due to lack of convergence, inadequate effective sample sizes and/or high levels of autocorrelation), ANOVA showed contradictory high heritability estimates.

Fixed effects of sex, experimental year and parental source population were tested by comparing likelihood ratios (ASSOC, WOMBAT) and DIC value (MCMCglmm) obtained for extended models run with and without the given effect (Supplementary material, Table S7. Accounting for experimental year and population effects sequentially increased the overall model support across both ASSOC and WOMBAT, while lower model fit was obtained by inclusion of population in MCMCglmm analysis. On the other hand, including sex as fixed effect resulted in less fitted models (indicated by higher maximum log likelihoods or DIC values obtained) across almost all WOMBAT and MCMCglmm analyses. Models both with and without sex effect were thus further explored in order to evaluate broad influence of sexual dimorphism on heritability estimates obtained in this study.

Extended models accounting for all three tested effects, i.e. individual sex, experimental year and source population, showed generally lower but more consistent heritability estimates from those estimated under the simple model (Figure 13). No significant results were obtained using ASSOC analysis, however animal models implemented in WOMBAT and MCMCglmm showed moderate to high heritability estimates across all traits connected to head size. Highest heritability was recorded for snout length (SnLgh; mean value of h^2 = 0.44), followed by head height (HHght; mean value of h^2 = 0.38), head length (HLgth; mean value of h^2 = 0.35), head width (HWdth; mean value of h^2 = 0.34), as well as lower jaw length (LwJaL; mean value of h^2 = 0.25) and lower jaw outlever (LwJaO; h^2 = 0.25). Significant but small heritability estimate was also recorded for length to hip (LtHip; mean value of h^2 = 0.15) in both WOMBAT and MCMCglmm animal models. No significant results were obtained for bite force (BiteF) and tail length (TailL) traits in any of the analyses employed.

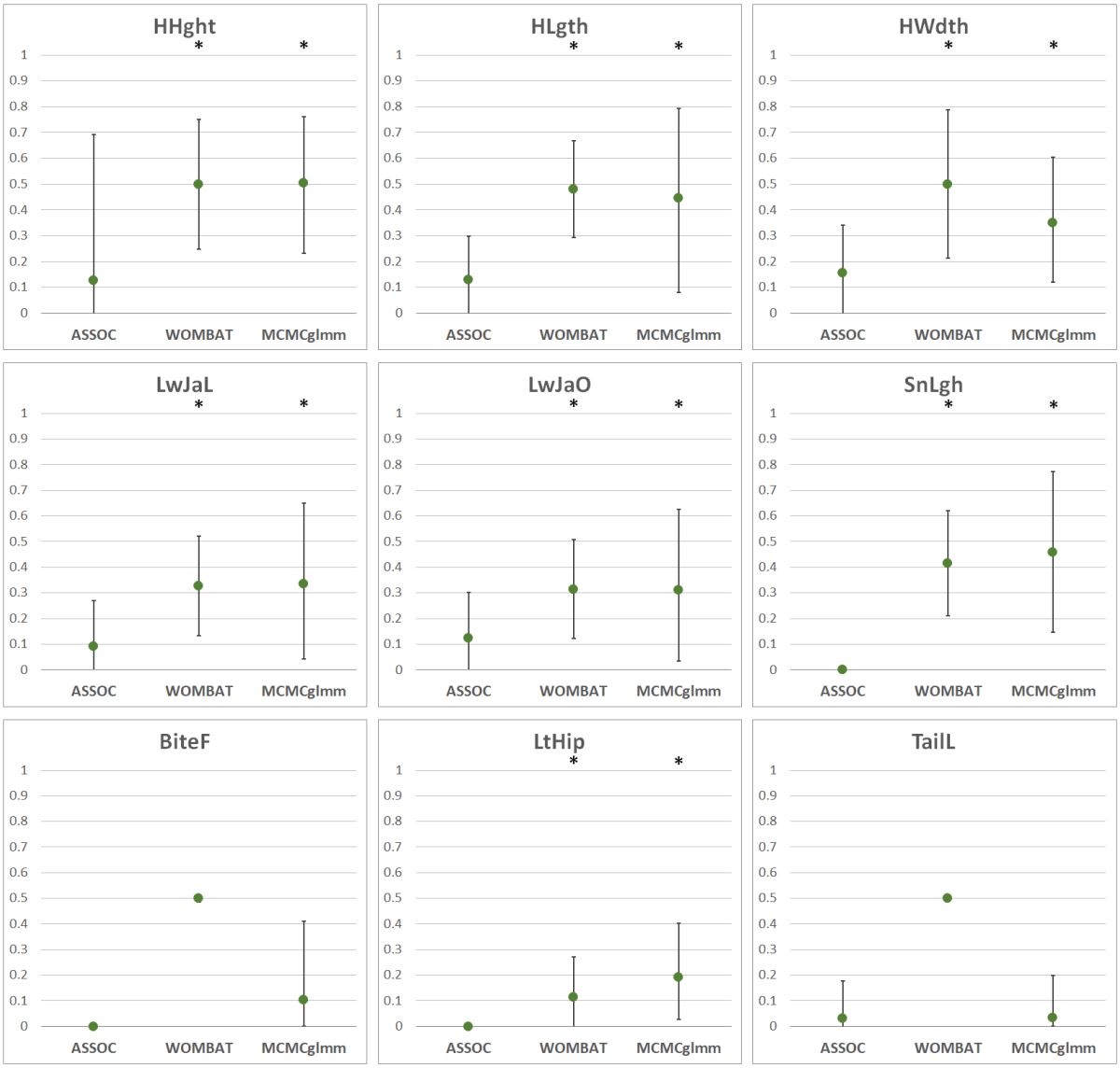


Figure 13 Heritability and associated standard errors (i.e. 95% credible intervals in Bayesian MCMCglmm analysis) estimated under the extended model with sex, experimental year and source population as fixed effects. Asterisks mark analyses that were significant with $p<0.05$ (ASSOC) and models with small autocorrelation which converged successfully (WOMBAT, MCMCglmm). Phenotypic trait abbreviations are defined in the text.

Extended models accounting only for year and source population showed much higher heritability estimates than results obtained using simple models or extended model including sex as fixed effect (Figure 14). Notably, high heritability values and good model support were once more attained for both head width (Hwdth) and head height (HHght) across all three analyses, with mean heritability values of 0.66 and 0.59, respectively. Although no convergence was achieved in ASSOC models for the rest of the traits, WOMBAT and MCMCglmm analyses performed better. High heritability estimates were obtained for head

length (HLgth; mean value of h^2 = 0.75), snout length (SnLgh; mean value of h^2 = 0.67), and lower jaw outlever (LwJaO; mean value of h^2 = 0.59) using both models. Lower jaw length (LwJaL) showed equally high heritability estimate of 0.64 in WOMBAT, but no significant results were obtained in MCMCglmm analysis. Similar increase in heritability estimates was also obtained for length to hip (LtHip), with mean heritability rising to 0.15 in WOMBAT and MCMCglmm analyses. Phenotypic measures of bite force (BiteF) and tail length (TailL) once again showed extremely low and inconclusive heritability estimates.

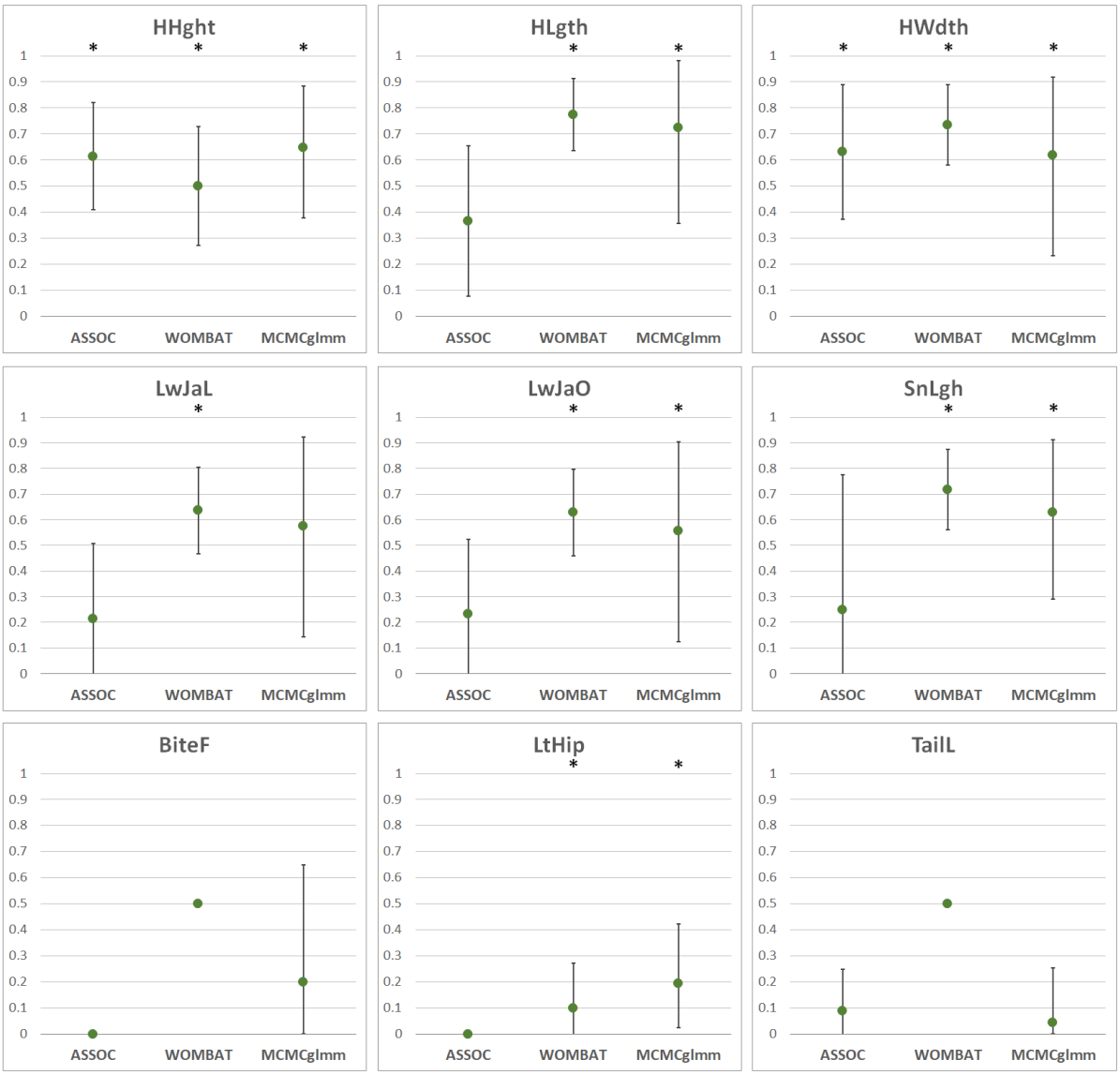


Figure 14 Heritability and associated standard errors (i.e. 95% credible intervals in Bayesian MCMCglmm analysis) estimated under the extended model with only experimental year and source population as fixed effects. Asterisks mark analyses that were significant with p<0.05 (ASSOC) and models with small autocorrelation which converged successfully (WOMBAT, MCMCglmm). Phenotypic trait abbreviations are defined in the text.

4.2 Genomic patterns in wild populations

4.2.1 Genomic diversity

No significant deviations from Hardy-Weinberg equilibrium were detected in natural populations. Observed and expected heterozygosities within populations were similar and always in the same order of magnitude, while allelic richness followed the same trend as heterozygosity among different populations (Table 6).

Table 6 Genomic diversity indices estimated for wild *Podarcis* populations (n – number of samples; Ho – observed heterozygosity, He – expected heterozygosity, Ar – allelic richness, 95% CI (Ar) – 95% confidence interval for Ar, F_{IS} – inbreeding coefficient, 95% CI (F_{IS}) – 95% confidence interval for F_{IS}). Population abbreviations are defined in Figure 5.

Population	n	Ho	He	Ar	95% CI (Ar)	F_{IS}	95% CI (F_{IS})
P. siculus							
BJ	19	0.011	0.013	1.041	[1.037 - 1.045]	0.098	[0.048 - 0.093]
DU	25	0.047	0.055	1.149	[1.139 - 1.154]	0.128	[0.086 - 0.126]
KL	22	0.042	0.048	1.141	[1.135 - 1.147]	0.093	[0.052 - 0.091]
KP	30	0.025	0.029	1.100	[1.085 - 1.122]	0.110	[0.080 - 0.114]
OB	25	0.065	0.084	1.238	[1.226 - 1.248]	0.206	[0.154 - 0.207]
OS	25	0.055	0.063	1.168	[1.161 - 1.174]	0.097	[0.051 - 0.096]
PG	20	0.038	0.044	1.170	[1.097 - 1.270]	0.078	[0.032 - 0.111]
PJ	20	0.029	0.041	1.131	[1.115 - 1.144]	0.226	[0.179 - 0.230]
PK	43	0.025	0.027	1.086	[1.074 - 1.121]	0.044	[0.021 - 0.054]
PM	46	0.023	0.024	1.076	[1.070 - 1.084]	0.070	[0.046 - 0.070]
RK	25	0.060	0.076	1.213	[1.199 - 1.220]	0.191	[0.151 - 0.182]
SC	19	0.025	0.03	1.096	[1.085 - 1.103]	0.141	[0.090 - 0.134]
ST	18	0.056	0.081	1.242	[1.223 - 1.257]	0.252	[0.194 - 0.250]
VC	25	0.055	0.067	1.189	[1.177 - 1.197]	0.155	[0.106 - 0.159]
P. melisellensis							
BD	19	0.032	0.043	1.127	[1.115 - 1.139]	0.219	[0.173 - 0.227]
BR	28	0.017	0.024	1.079	[1.064 - 1.100]	0.186	[0.154 - 0.217]
GL	10	0.016	0.025	1.072	[1.065 - 1.078]	0.279	[0.192 - 0.280]
GN	20	0.038	0.049	1.144	[1.133 - 1.156]	0.175	[0.122 - 0.187]
JK	20	0.011	0.016	1.050	[1.044 - 1.054]	0.242	[0.202 - 0.256]
KM	15	0.007	0.009	1.028	[1.024 - 1.030]	0.186	[0.146 - 0.193]
MB	20	0.004	0.005	1.022	[1.015 - 1.029]	0.094	[0.064 - 0.110]
PZ	20	0.023	0.034	1.102	[1.092 - 1.109]	0.262	[0.210 - 0.261]
RV	20	0.024	0.037	1.118	[1.102 - 1.133]	0.281	[0.244 - 0.295]
SN	17	0.029	0.046	1.136	[1.125 - 1.145]	0.301	[0.236 - 0.304]
VB	29	0.057	0.072	1.295	[1.140 - 1.480]	0.110	[0.076 - 0.138]
VT	20	0.010	0.014	1.044	[1.039 - 1.048]	0.216	[0.171 - 0.241]

In *P. siculus* populations, diversity indices were highest in mainland population (ST) and islands in close proximity to mainland (OB, RK, OS, VC, DU and KL) (Ho = 0.042–0.065, Ar = 1.141–1.242). Median diversity values were recorded for one other population near mainland (PJ; Ho = 0.029, Ar = 1.170), and another one situated furthest away from it (PG; Ho = 0.038, Ar = 1.295). Islands near Lastovo (BJ, KP, PK, PM, SC) exhibited lowest diversity indices among *P. siculus* populations (Ho = 0.011–0.025, Ar = 1.041–1.100). As expected, diversity was lower in Pod Mrčaru (PM) than Pod Kopište (PK) population (Ho = 0.023, Ar = 1.076 vs. Ho = 0.025, Ar = 1.086, respectively). However, no evidence of increased inbreeding was found in Pod Mrčaru population (F_{IS} = 0.070).

In general, *P. melisellensis* populations exhibited less genomic diversity than *P. siculus*. Particularly low genetic diversity was recorded for Mali Barjak (MB), three populations inhabiting volcanic islands (JK, KM and BR) and two islands near Lastovo (GL and VT) (Ho = 0.004–0.017, Ar = 1.022–1.079). Mainland population (SN; Ho = 0.029, Ar = 1.136) did not show higher diversity than insular populations concentrated around Vis (BD, GN, PZ, RV; Ho = 0.023–0.038, Ar = 1.102–1.144). Highest diversity estimates were obtained for *P. melisellensis* population from Veli Barjak (VB; Ho = 0.057, Ar = 1.295).

Low to median inbreeding was detected for all sites (F_{IS} = 0.044 – 0.301). Genomic diversity was not correlated with island area in either *P. siculus* or *P. melisellensis* populations (Pearson's r for all indices < 0.32).

4.2.2 Genomic differentiation

As expected for different species comparison, extremely high genomic differentiation was recorded between *P. siculus* and *P. melisellensis* populations (pair-wise F_{ST} > 0.89; Figure 15A). Among *P. siculus* populations, highest F_{ST} values were recorded between mainland and northern islands (ST, DU, OB, OS, RK, VC), henceforth referred to as 'Split' group, and the rest of the populations, termed 'Lastovo' group (BJ, KP, PG, PK, PM, SC, as well as PJ and KL) (F_{ST} = 0.53–0.78; Figure 15B). Within 'Split' group, Oštrica (OS) and Veli Dupinić (DU) showed highest differentiation from other populations (F_{ST} = 0.29–0.44). Among 'Lastovo' group Pod Kopište (PK), Pod Mrčaru (PM), Kopište (KP), and Sušac (SC) were highly similar (F_{ST} = 0.04–0.16). Kluda (KL) and Pijavica (PJ) were less differentiated from 'Split' group than other 'Lastovo' populations (F_{ST} = 0.53–0.68 vs. F_{ST} = 0.57–0.78). Due to their geographic proximity, these results point towards possible introgression of 'Split' group in *P. siculus* populations on Kluda (KL) and Pijavica (PJ) islands.

Low but significant genomic differentiation was recorded between Pod Mrčaru and Pod Kopište *P. siculus* populations (F_{ST} = 0.04).

Podarcis melisellensis populations however, showed higher levels of intra-species genetic differentiation (Figure 15C). For instance, extremely high genomic divergence was recorded between Mali Barjak (MB), volcanic islands (JK, KM, BR), and mainland and insular populations near Lastovo (SN, GL, VT) (F_{ST} = 0.74–0.93). On the other hand, islands in Vis archipelago (GN, PZ, BD, RV, VB) showed low levels of genomic divergence (F_{ST} = 0.10–0.34). Veli Barjak (VB) in general showed lowest pairwise F_{ST} values with other *P. melisellensis*, but also with *P. siculus* populations (Figure 15A).

All population pairwise F_{ST} calculations were significant (p-values = 0). Population pairwise exact F_{ST} values are included in the Supplementary materials (Table S8).

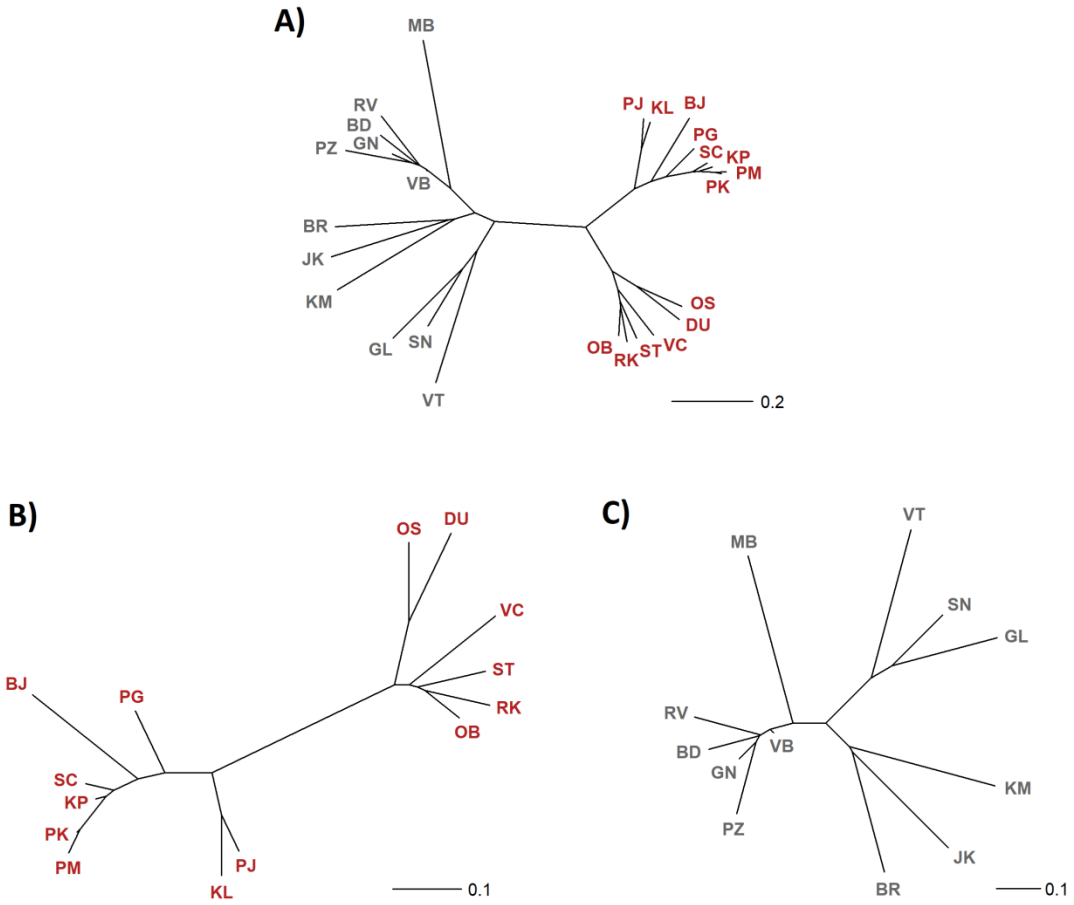


Figure 15 Neighbour-joining trees based on pairwise F_{ST} values for **A)** all 26 wild *Podarcis* populations, **B)** 14 wild *P. siculus* populations, and **C)** 12 wild *P. melisellensis* populations. *Podarcis siculus* populations are marked in red and *P. melisellensis* populations in black colour. Population abbreviations are defined in Figure 5.

PCA analysis of genomic dataset containing both species revealed strong species separation along first principal component, explaining 69.8% of total genomic variation (Figure 16A). Second principal component described 7.98% of the total variation, and pointed towards separation of 'Split' group (ST, DU, OB, OS, RK, VC) from other *P. siculus* populations. Additionally, several individuals from Veli Barjak (VB) and one individual from Mala Palagruža (PG) showed clear differentiation from others of the same population, and more similarity with the other species.

Due to this extreme variability among some individuals, and in order to detect broader patterns of genomic separation within each species, several outlier samples were filtered out prior to the PCA analysis of *P. siculus* and *P. melisellensis* datasets (Figure 16B and 16C). Two individuals, one from Mala Palagruža (PG) and one from Kopište (KP), were removed from the PCA analysis of *P. siculus* populations, and two individuals from Veli Barjak (VB) were removed from the analysis of *P. melisellensis* populations.

Subsequent PCA analysis of *P. siculus* populations highlighted already observed separation between 'Split' and 'Lastovo' group (BJ, KP, PG, PK, PM, SC, PJ, KL) along first principal component, which explained 25.12% of total variance (Figure 16B). Second principal component (explaining 4.49% of variance) emphasised differentiation of Oštrica (OS) and Veli Dupinić (DU) populations from the rest of the 'Split' group. Populations from Pijavica (PJ) and Kluda (KL), islands closest to mainland site of Split (ST), once more showed more similarity with 'Lastovo' group.

First principal component in PCA analysis of *P. melisellensis* dataset explained 12.93 % of total genomic variance and pointed towards separation of mainland (SN) and two islands near Lastovo (GL and VT) from other populations (Figure 16C). Second principal component explained 7.83% variance and mostly contributed to differentiation between volcanic islands (BR, KM, and JK) and islands in Vis archipelago (BD, GN, PZ, MB, VB). Veli Barjak (VB) population again showed extreme inter-population genomic variability.

PCA analysis on genomic dataset containing only Pod Mrčaru and Pod Kopište *P. siculus* populations showed clear genomic differentiation between Pod Mrčaru and Pod Kopište populations on first principal component, which explained 3.83% of total genomic variance (Figure 17).

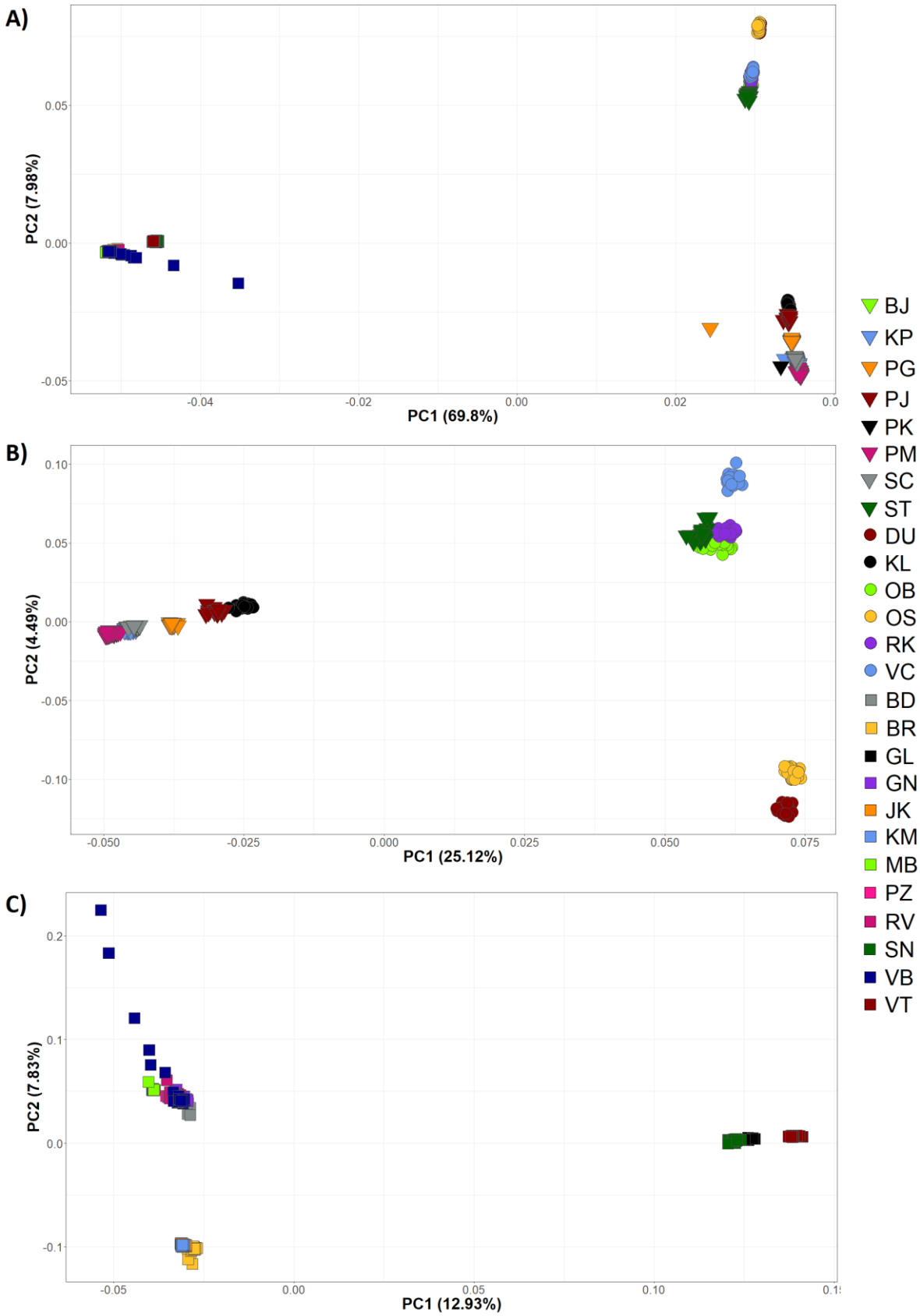


Figure 16 PCA analyses of **A)** all 26 wild *Podarcis* populations, **B)** 14 wild *P. siculus* populations, and **C)** 12 wild *P. melisellensis* populations. Population abbreviations are defined in Figure 5.

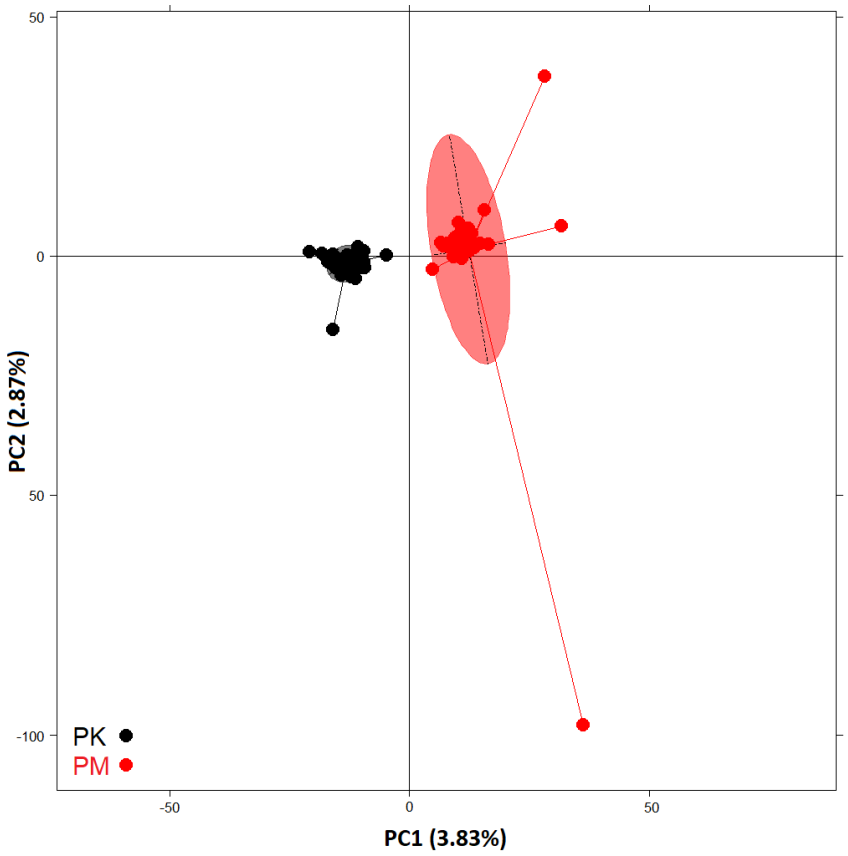


Figure 17 PCA analysis of Pod Mrčaru (PM) and Pod Kopište (PK) *P. siculus* populations.

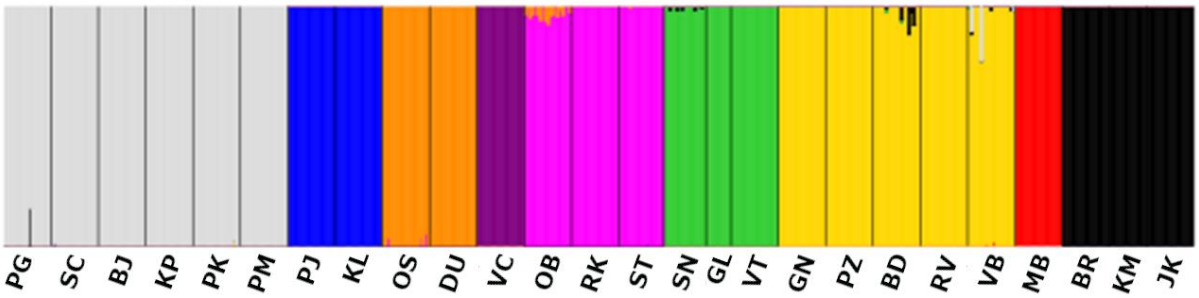
Isolation by distance was significant in both *P. siculus* (Mantel r = 0.72; p = 0.001) and *P. melisellensis* populations (Mantel r = 0.61; p= 0.001) (Supplementary material, Figure S1).

Model complexity that maximized marginal likelihood in *fastStructure* analysis of all 26 *Podarcis* populations identified nine genomic clusters (K=9) (Figure 18A). Five of those clusters were described among *P. siculus* populations. 'Lastovo' group separated into two clusters, with two small islets close to the coast (PJ, KL) showing different genomic background than the rest. Three additional genomic clusters were identified among populations from 'Split' *P. siculus* group – Visovac (VC) formed a separate cluster by itself; and Oštrica (OS) and Veli Dupinić (VC) composed another; with mainland (ST) and two remaining northern islands (OB, RK) grouped together in the last one. Among *P. melisellensis* populations there were four main clusters identified: first one consisted of mainland population (SN) and two islands near Lastovo (VT, GL); second one contained three volcanic islands (JK, KM, BR); third one only Mali Barjak (MB) population; and fourth

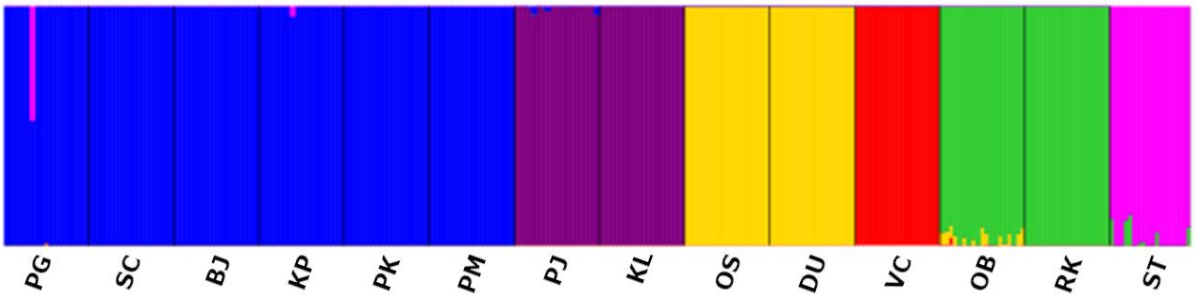
grouped together the remaining islands located in the Vis archipelago (GN, PZ, BD, RV, VB).

Analysed separately, *P. siculus* and *P. melisellensis* datasets showed higher degree of population structuring. Models that best explained the structure in data were those with K=6 and K=5 for *P. siculus* and *P. melisellensis*, respectively. In addition to the structure described above (obtained using the dataset with both species), these models identified *P. siculus* population from mainland (ST) and *P. melisellensis* population from Veli Tajan (VT) as separate genomic clusters, divergent from other populations of the same species (Figure 18B,C).

A) all *Podarcis* populations (K=9)



B) *P. siculus* populations (K=6)



C) *P. melisellensis* populations (K=5)

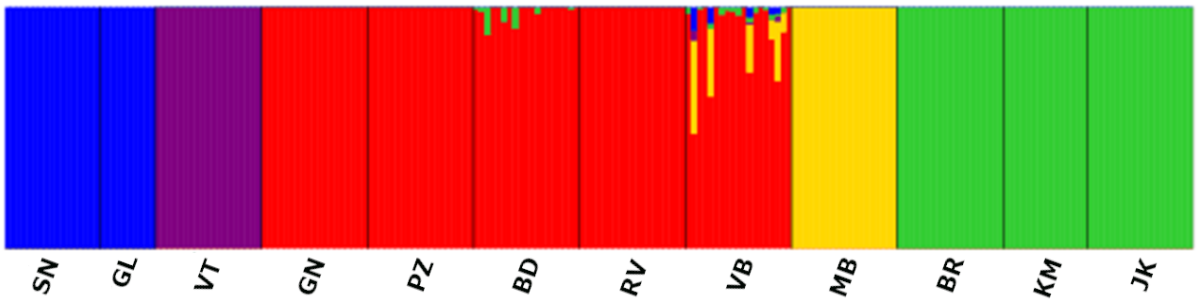


Figure 18 *fastStructure* results for **A)** all 26 wild *Podarcis* populations (K=9), **B)** 14 wild *P. siculus* populations (K=6), and **C)** 12 wild *P. melisellensis* populations (K=5). Population abbreviations are defined in Figure 5.

Most of the population assignments to identified clusters aligned with the pair-wise F_{ST} values and PCA clusters obtained previously. Small degree of admixture was found between *P. siculus* and *P. melisellensis* populations. Notably, *P. melisellensis* from Veli Barjak (VB) showed a certain degree of admixture with *P. siculus* populations from Lastovo area (Figure 18A). In addition to this, one *P. siculus* individual from Mala Palagruža (PG) seemed to share some genomic background from *P. melisellensis* genomic cluster formed by volcanic islands population (JK, KM, BR; Figure 18A). However, in the analysis using only *P. siculus* populations, this introgression in Mala Palagruža (PG) appeared to be from the genomic type found in mainland population of Split (ST; Figure 18B).

As expected, hierarchical AMOVA revealed that most of the variation (84.16%) across all analysed SNPs best described the genomic difference between the two species (Supplementary materials, Table S9). When species were analysed separately, AMOVA results supported population structure obtained in *fastStructure* analyses, with the biggest partition of genomic variance (56.02% for *P. siculus* and 50.8% for *P. melisellensis*) found among identified genomic clusters (Supplementary materials, Tables S10 and S11). Both species also showed high variation within individuals (30.41 % for *P. siculus* and 22.54 % for *P. melisellensis*), while smaller variation was found among individuals within populations (6.37% and 8.79% for *P. siculus* and *P. melisellensis* respectively). Variation among populations within genomic clusters was higher for *P. melisellensis* (17.87%) than for *P. siculus* (7.2%).

4.3 Identification of loci under selection

4.3.1 Genome scans for loci putatively under selection

Only one locus was identified as an outlier in BayeScan analysis of Pod Mrčaru and Pod Kopište populations (Supplementary material, Figure S2). However, genome scans implemented in Arlequin and *PCAdapt* performed better, identifying 53 and 90 outlier loci putatively under selection, respectively (Supplementary material, Figures S3 and S4). Roughly half of the outlier loci identified in *PCAdapt* were significant in Arlequin analysis as well (Figure 19). Additional 367 loci were identified as contributing the most to Pod Mrčaru – Pod Kopište separation in standard PCA analysis of allele frequencies using *adegenet* (Supplementary material, Figure S5). The loci exhibiting highest loadings on PC1

encompassed all those found significant in genome scans analyses, along with 270 additional loci not detected by those methods (Figure 19).

46 loci in total (45 loci identified as significant in Arlequin, *PCAdapt* and PCA analysis, and one additional locus significant in all four analyses, including BayeScan) were considered candidate loci for selection in Pod Mrčaru *P. siculus* population (Figure 19). These loci represent 1.9% of all SNPs polymorphic in the dataset with only Pod Mrčaru and Pod Kopište populations and 0.23% of all 19550 loci from the full dataset consisting of all 26 wild *Podarcis* populations (Table 4).

Loci specific F_{ST} values obtained from Arlequin genome scan analysis are provided in Supplementary material, Figure S6.

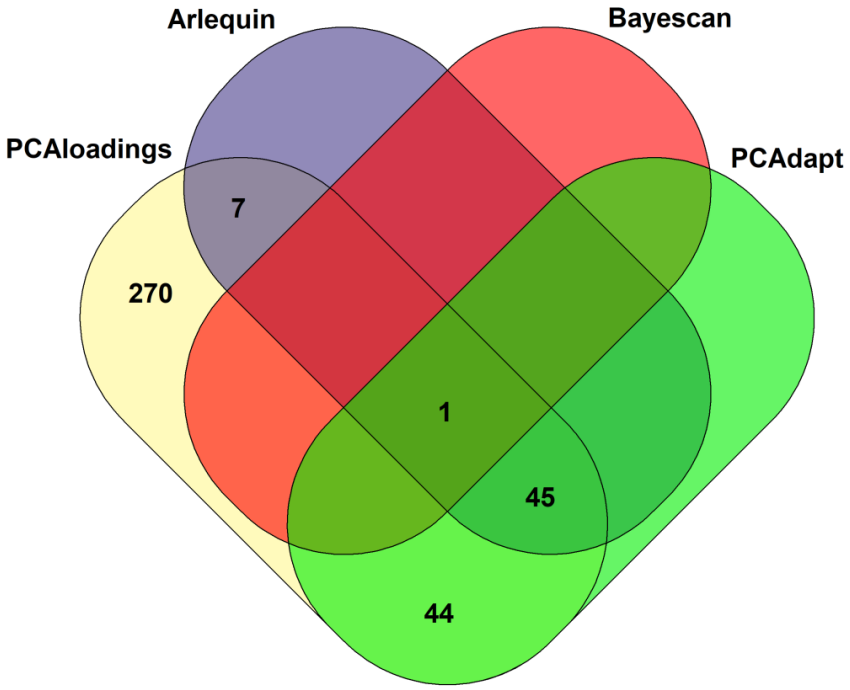


Figure 19 Venn diagram of candidate loci for selection in Pod Mrčaru and Pod Kopište populations inferred using BayeScan, Arlequin, *PCAdapt*, and *adegenet* method based on PCA loadings (PCAloadings).

4.3.2 Phenotypic differentiation and genotype-phenotype associations

PCA analysis of all sampled individuals from both species revealed that more phenotypic differentiation exists between male and female individuals, then between lizards from different species (Supplementary material, Figure S7). The trend highlights high sexual

dimorphism found in these two species, but persists even when sexes are analysed separately (Supplementary material, Figure S8), indicating low level of phenotypic differentiation and general overlap of phenotypic space between *P. siculus* and *P. melisellensis* in the investigated area. Results of PCA analyses conducted on *P. siculus* and *P. melisellensis* populations separately, showed high variability and no clear pattern of populations divergence due to phenotype (Supplementary material, Figures S9 and S10). Due to the lack of any discernible trend among numerous populations analysed together, subsequent analyses focused only on Pod Mrčaru and Pod Kopište *P. siculus* individuals.

High phenotypic differentiation was recorded for several traits in P_{ST} analyses of both female and male individuals from Pod Mrčaru and Pod Kopište. In particular, snout-vent length (SVLgh), as well as hind (HLLgh), front (FLLgh), and inter limb length (ILLgh), were highly differentiated in both sexes ($P_{ST} > 0.9$; Figure 20). Lower estimates of P_{ST} values in those traits highly exceed differentiation expected under the neutral model ($P_{ST} > F_{ST}$), implying influence of divergent selection. High phenotypic differentiation was also recorded for body mass (BMs; $P_{ST} = 0.79$) in females. On the other hand, the confidence of P_{ST} estimation for BMs in male individuals was too low to draw any conclusions about selection influence in its differentiation.

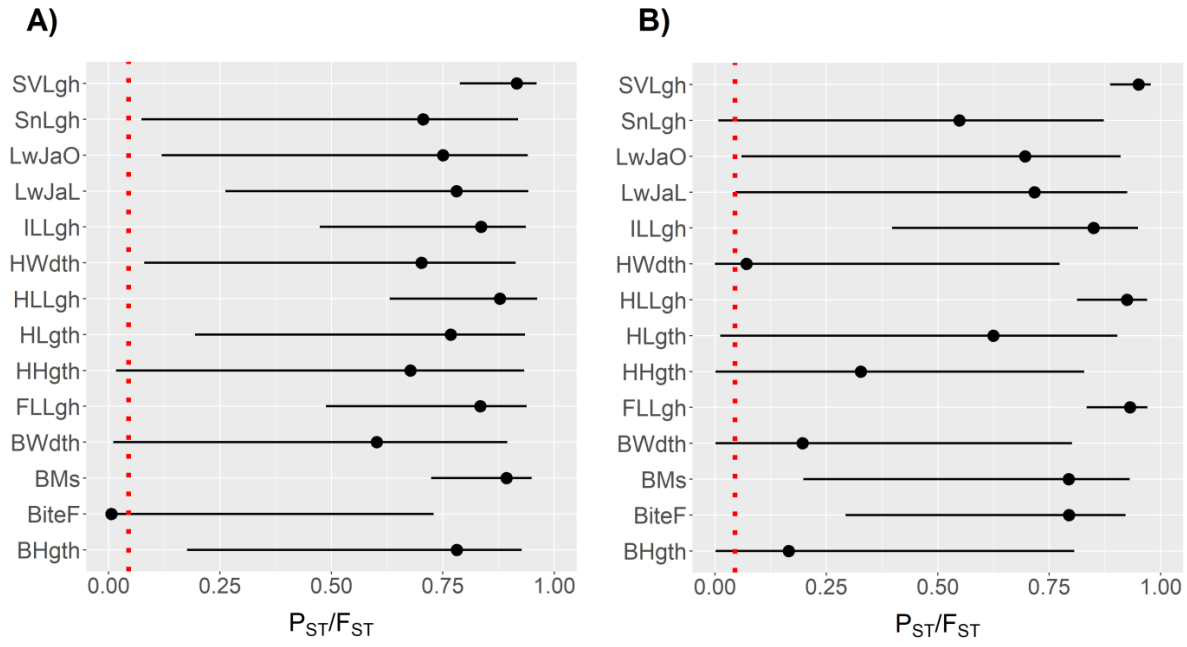


Figure 20 Estimated P_{ST} values (black dots) and corresponding confidence intervals (whiskers) compared to population pairwise F_{ST} value (red dashed line) for **A)** female and **B)** male *P. siculus* individuals from Pod Kopište and Pod Mrčaru populations. Phenotypic trait abbreviations are defined in Table 1.

All other analysed phenotypic traits showed similarly wide range of P_{ST} confidence intervals, hindering accurate interpretation of the results. However, it's interesting to note that extremely low P_{ST} values ($P_{ST} < 0.1$; indicative of balancing selection) were suggested for bite force (BiteF) in female, and head width (HWdth) in male individuals, while the analysis of opposite sex showed relatively high phenotypic differentiation in the same traits ($P_{ST} > 0.7$; indicating directional selection). Obtained P_{ST} values were extremely similar across all tested c/h^2 parameter values for both female and male datasets (Supplementary material, Figures S11 and S12).

Employing the LFMM analysis on individuals from all investigated *P. siculus* or *P. melisellensis* populations resulted in extremely high GIF values (GIF > 3) obtained for most of the analysed traits and low model fit due to extreme deviation from expected p-value distribution. LFMM genotype-phenotype association analyses were therefore likewise performed using only individuals from Pod Mrčaru and Pod Kopište. These analyses showed expected genomic inflation factor (GIF) value close to 1 for all analysed traits. A visual inspection of p-values histograms, however, indicated that obtained GIF values were overly conservative across all variables. Modified GIFs were therefore used to obtain a more uniform distribution of p-values (Supplementary material, Table S12; Figures S13 and S14).

Considerably more loci were found associated with analysed phenotypic traits in LFMM analysis of male, than in female individuals from Pod Mrčaru and Pod Kopište (Figure 21). Males showed high number of loci associated with almost all investigated traits, particularly those connected to head size (LwJaL, no. loci = 53; HLgth, no. loci = 49; LwJaO, no. loci = 46; HWdth, no. loci = 40; and SnLgh, no. loci = 39), as well as limb length (HLLgh, no. loci = 41; ILLgh, no. loci =41; FLLgh, no. loci = 34) and body mass (BM, no. loci = 52). For females, highest number of loci was found associated with bite force (BiteF, no. loci = 31), head length (HLgth, no. loci = 28) and inter limb length (ILLgh, no. loci = 25).

Some of the detected loci showed significant association with several phenotypic traits for a given sex, which was expected due to high correlation between tested measures, especially those related to head size and shape. However, only 1.4% of the identified loci of interest in LFMM analyses were shared between sexes (Figure 21). Overall, 285 unique loci were considered associated with different phenotypic traits in LFMM analyses of female and male *P. siculus* individuals.

High overlap was found between loci showing association with female or male phenotype and 46 identified as putatively under selection in the two focal populations (Figure 21). Specifically, 17 out of the 285 unique loci detected by LFMM genotype-phenotype association analysis were also considered candidates for selection in Pod Mrčaru and Pod Kopište *P. siculus* populations.

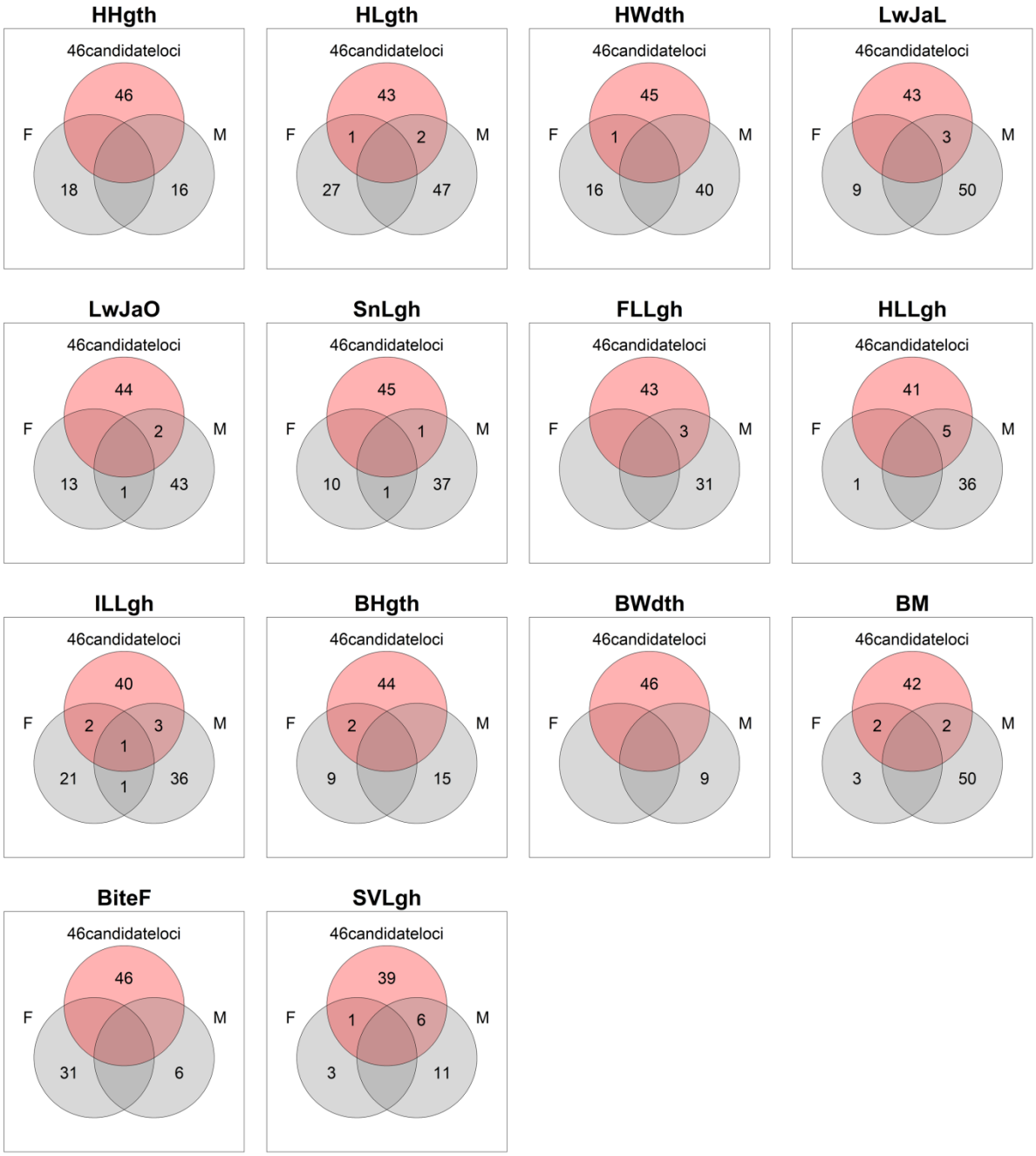


Figure 21 Significant loci associated with each phenotypic trait in LFMM analysis of female (F) and male (M) individuals, and their overlap with candidate loci putatively under selection in Pod Kopište and Pod Mrčaru populations (46candidateloci). Phenotypic trait abbreviations are defined in Table 1.

4.3.3 Genotype-environment associations

RDA analysis of genotype-environment associations in *P. siculus* populations was based on 2 Moran's eigenvectors (dbMEMs) and first 3 principal components obtained from PCA analysis of 21 ecological variables (which together explained 90.9% of all ecological variance, Supplementary material Figure S15). Variance partitioning between full RDA model and two partial RDA analyses conducted using all polymorphic loci in *P. siculus* dataset, revealed that 8.86% of total genomic variance could be explained by ecological factors after controlling for spatial structure (Table 7). Geographical distance on the other hand accounted for 5.24% of genomic variance after controlling for ecological variation among sampling sites. The joint influence of ecological and geographical components was high and accounted for 13.15% of total genomic variance, which is not surprising due to the high correlation observed between PC1 and dbMEM1 variables (Pearson's r = -0.91). Orthogonal projection of RDA scores from partial analysis of ecological variables after controlling for spatial structure showed clear separation of populations according to genomic clusters inferred previously, with first RDA axis accounting for 58.14%, and second for 32.74% of explained variance (Figure 22).

Table 7 RDA variance partitioning results for *P. siculus* populations across all genomic markers, *P. siculus* populations using only 46 candidate markers under selection in Pod Mrčaru and Pod Kopište populations, and *P. melisellensis* dataset with all genomic markers. R^2 *adjusted* equals percentage of total variance; *% inertia* is percentage of explained (constrained) variance. Significance was determined using ANOVA after 999 permutations.

Model	Predictors/effect	R^2 adjusted	% inertia	Significance
P. siculus all				
RDA full	Ecology + geography	0.27257	1	p=0.001
pRDA ecology	Ecology | geography	0.08864	0.33329	p=0.001
pRDA geography	Geography | ecology	0.05241	0.19816	p=0.001
-	Ecology ∩ geography	0.13152	0.46855	-
P. siculus 46 candidate loci				
RDA full	Ecology + geography	0.35739	1	p=0.001
pRDA ecology	Ecology | geography	0.14125	0.39807	p=0.001
pRDA geography	Geography | ecology	0.08664	0.24428	p=0.001
-	Ecology ∩ geography	0.1295	0.35765	-
P. melisellensis				
RDA full	Ecology + geography	0.17616	1	p=0.001
pRDA ecology	Ecology | geography	0.06798	0.39123	p=0.001
pRDA geography	Geography | ecology	0.06935	0.39839	p=0.001
-	Ecology ∩ geography	0.03883	0.21038	-

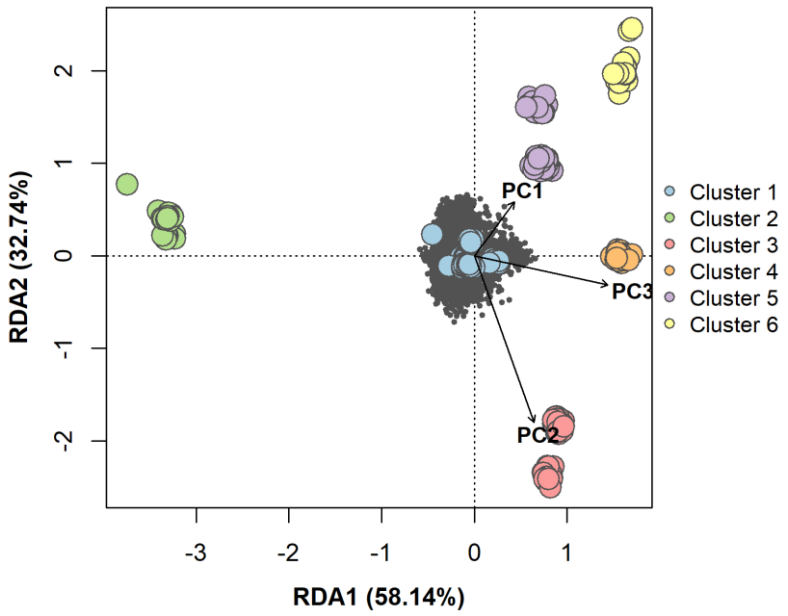


Figure 22 RDA triplot obtained from partial analysis of all genomic markers (grey dots) and ecological factors (arrows) after controlling for spatial structure in *P. siculus* populations (Cluster 1 = PG, SC, BJ, KP, PK, PM; Cluster 2 = PJ, KL; Cluster 3 = OS, DU; Cluster 4 = VC; Cluster 5 = OB, RK; Cluster 6 = ST; population abbreviations are defined in Figure 5).

In RDA analyses of *P. siculus* populations using only 46 candidate loci identified as under selection in Pod Mrčaru and Pod Kopište populations (see section 4.3.1 Genome scans for loci putatively under selection), 14.13% of variance could be explained by ecological variables, while geography accounted for 8.66% of total genomic variance (Table 7). Joint influence of ecological and geographical factors was again high and accounted for 12.95% of total variance in 46 candidate loci. RDA plots showed clear separation of Pod Mrčaru population across first two RDA axes, which accounted for 61.63% and 24.72% of explained variation respectfully (Figure 23).

RDA analysis of *P. melisellensis* genomic dataset was conducted using scores from first two principal components from PCA analysis of ecological variables (explaining 92.06% of all variance, Supplementary material Figure S16) and 2 dbMEM s that showed positive Moran's I values. RDA variance partitioning revealed that ecological factors explained 6.8% of total genomic variance in the dataset (Table 7). However, spatial component was marginally higher and accounted for 6.93% of total variance across populations, while 3.88% variance was explained by joint influence of ecological and geographical factors. RDA plots did not show clear separation according to previously inferred genomic structure (Figure 24).

All explored RDA models, axes and marginal effects were significant with p = 0.001.

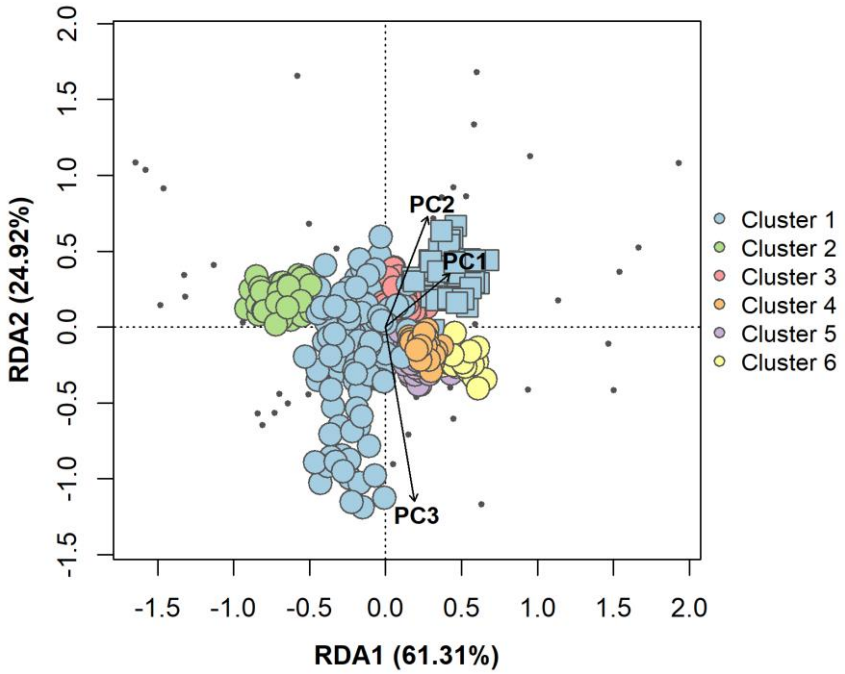


Figure 23 RDA triplot obtained from partial analysis of 46 candidate genomic markers (grey dots) for selection in Pod Mrčaru population and ecological factors (arrows) after controlling for spatial structure across all *P. siculus* populations (Cluster 1 = PG, SC, BJ, KP, PK, PM; Cluster 2 = PJ, KL; Cluster 3 = OS, DU; Cluster 4 = VC; Cluster 5 = OB, RK; Cluster 6 = ST; population abbreviations are defined in Figure 5). PM population is marked with squares.

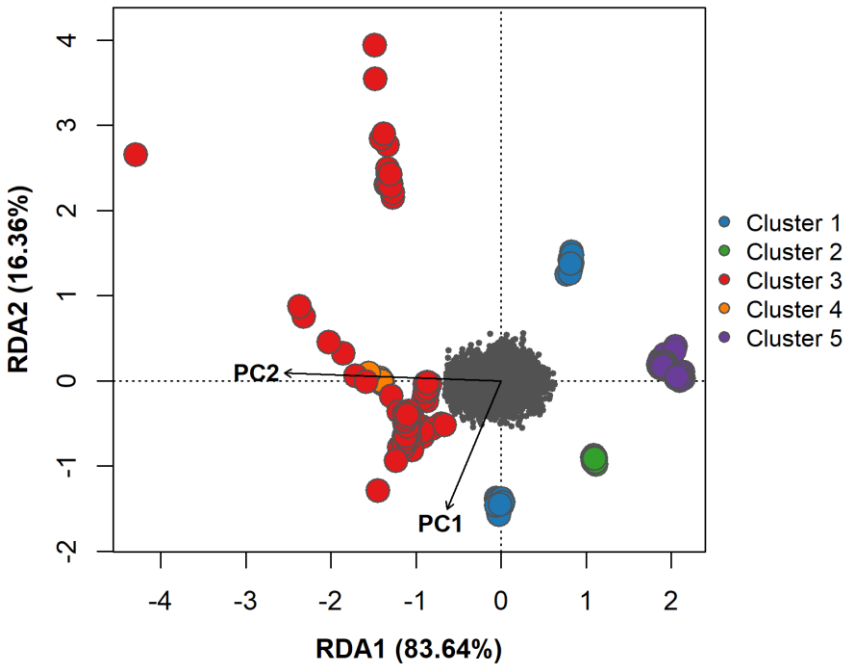


Figure 24 RDA triplot obtained from partial analysis of all genomic markers (grey dots) and ecological factors (arrows) after controlling for spatial structure in *P. melisellensis* populations (Cluster 1 = SN, GL; Cluster 2 = VT; Cluster 3 = GN, PZ, BD, RV, VB; Cluster 4 = MB; Cluster 5 = BR, KM, JK; population abbreviations are defined in Figure 5).

Univariate genotype-environment associations, inferred for each of the 23 environmental factors analysed, varied substantially across both species and Bayesian GEA approaches tested. In GEA analyses of *P. siculus* populations, large number of significant loci was found across all three Bayesian methods, however little overlap was detected among the analyses themselves (Table 8).

Table 8 Significant loci associated with each environmental factor obtained for 14 *P. siculus* populations using different Bayesian GEA methods, and their overlap with 46 loci identified as putatively under selection in Pod Mrčaru and Pod Kopište populations (marked in square brackets). Overlap of loci found significant across different GEA methods is noted in the last column (*one locus shared across 2 environmental variables, **one locus shared across 3 variables). Variable abbreviations are defined in Table 2.

Variable	Bayenv2	Baypass	BayeScEnv	GEA overlap
Longitude	215 [4]	254 [-]	465 [-]	4
Latitude	319 [3]	96 [-]	347 [-]	-
Area	67 [-]	166 [1]	317 [-]	-
Altitude	72 [1]	283 [1]	343 [-]	-
DistanceToLand	151 [2]	223 [4]	189 [-]	1
DistanceToLargeIsland	131 [-]	191 [4]	149 [-]	-
DepthToCoastMax	197 [2]	262 [2]	55 [-]	-
TemperatureMean	113 [2]	550 [21]	368 [-]	3*
TemperatureMin	86 [1]	365 [6]	250 [-]	-
TemperatureMax	161 [2]	158 [6]	81 [-]	-
TemperatureRange	157 [-]	135 [1]	66 [-]	-
PrecipitationMean	293 [3]	369 [4]	252 [-]	3
PrecipitationMin	403 [2]	239 [1]	208 [-]	3*
PrecipitationMax	270 [1]	341 [3]	122 [-]	-
PrecipitationRange	160 [1]	339 [3]	49 [-]	-
SolarRadiationMean	462 [2]	126 [-]	400 [-]	2**
SolarRadiationMin	328 [2]	163 [1]	378 [-]	1**
SolarRadiationMax	173 [2]	323 [3]	285 [-]	1
SolarRadiationRange	153 [-]	133 [-]	220 [-]	-
WindSpeedMean	349 [1]	124 [-]	348 [-]	-
WindSpeedMin	280 [2]	196 [2]	323 [-]	1**
WindSpeedMax	356 [1]	99 [-]	352 [-]	-
WindSpeedRange	458 [4]	58 [-]	382 [-]	-

Covariance matrices obtained from both Bayenv2 and Baypass methods showed high correlation to the matrix of *P. siculus* population pairwise F_{ST} values (Supplementary material, Figure S17), indicating adequate approximation of neutral population structure. Though relatively high overlap was recorded among significant loci detected in Bayenv2 and Baypass analyses (likely due to the similarity of applied algorithm), BayeScEnv pointed towards markedly different set of loci governing genotype-environment associations in *P. siculus* populations. Thus, only 16 unique SNPs were detected as significantly associated with the environment across all 3 Bayesian GEA analyses (Table 8). Four of them showed significant interaction with geographic latitude (Latitude), and six more were highly associated with two precipitation variables (PrecipitationMean and PrecipitationMin). Another four loci were identified in GEA analyses of mean annual temperature (TemeratureMean; one additional locus associated with TemperatureMean was already described for PrecipitationMin) and solar radiation (SolarRadiationMean). Two other unique SNPs were found significant for distance to mainland (DistanceToLand) and maximum solar radiation (SolarRadiationMax), while both minimum wind speed (WindSpeedMin) and solar radiation (SolarRadiationMin) showed association with SNPs previously identified in GEA analyses of SolarRadiationMean variable.

Using Baypass under the core model, 110 loci were further identified as putatively under selection in all *P. siculus* populations – 75 of them under directional selection and 35 under balancing selection (Supplementary material, Figure S18). Moreover, 6 of those loci were also identified as putatively under selection in Pod Mrčaru and Pod Kopište populations (see section 4.3.1 Genome scans for loci putatively under selection). The detected overlap between candidate loci for selection in the two focal populations and loci found under selection in Baypass core model analysis of all *P. siculus* populations was higher than could be expected purely by chance ($p<0.0001$).

Comparison of *P. siculus* GEA significant loci with 46 candidate loci under selection in Pod Mrčaru and Pod Kopište populations revealed some overlap with Bayenv2 and Baypass results, although none was found for BayeScEnv analysis (Table 8). Specifically, 4 unique loci were identified as important for Pod Mrčaru and Pod Kopište population divergence, and for genotype-environment associations in both Bayenv2 and BayPass analyses of all *P. siculus* populations (Figure 25). Those 4 loci showed significant interactions with 6 different environmental variables – 2 loci were highly associated with three temperature variables (TemperatureMax, TemperatureMean, TemperatureMin), one with both distance to mainland

(DistanceToLand) and precipitation (PrecipitationMean), and the last one with wind speed (WindSpeedMin) (Figure 25).

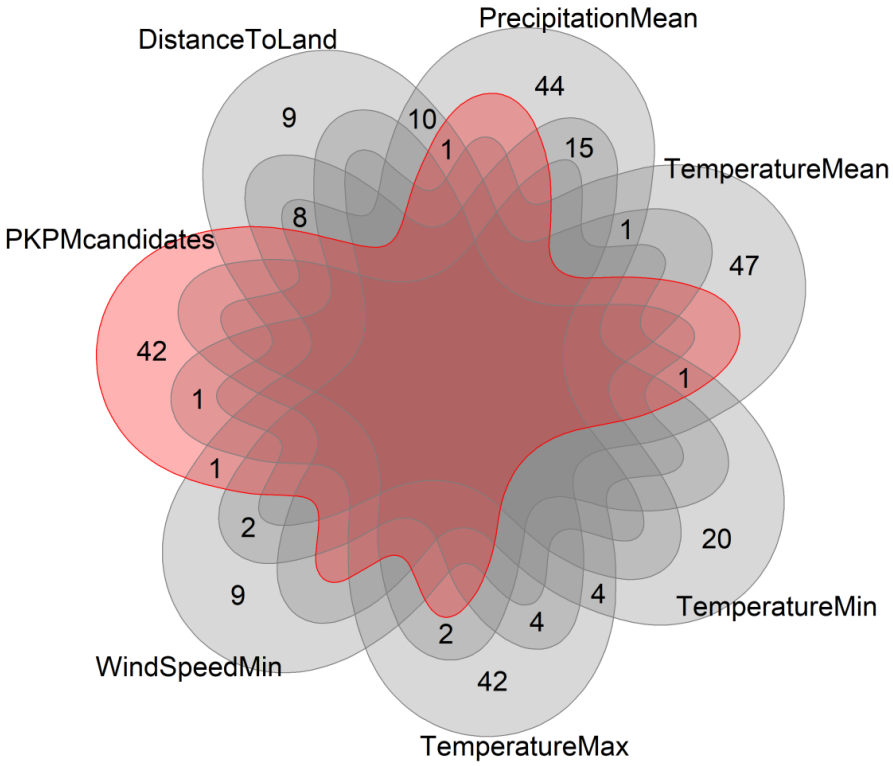


Figure 25 Overlap of candidate loci under selection in Pod Mrčaru and Pod Kopište populations (PKPMcandidates), and those showing significant associations with the environment in Bayenv2 and Baypass GEA analyses of all wild *P. siculus* populations. Variable abbreviations are defined in Table 2.

Extremely variable results were obtained from GEA analyses on *P. melisellensis* populations (Table 9). Bayenv2 method detected relatively low number of SNPs with high BF values indicative of a strong relationship with the environment. On the other hand, extremely high number of loci was found significantly associated with environmental factors in Baypass analysis. This is almost certainly due in part to a fallacious covariance matrix estimation in Baypass, which differed significantly from population pairwise F_{ST} values inferred previously and depicted Veli Barjak (VB) as highly differentiated from other populations (Supplementary material, Figure S19). Results from both Bayenv2 and Baypass analyses showed very little overlap with BayeScEnv. Nevertheless, 5 different loci were identified as significantly associated with distance to nearest large island (DistanceToLargeIsland) across all three analyses, with one of them showing additional strong interaction with mean annual wind speed (WindSpeedMean).

Table 9 Significant loci associated with each environmental factor obtained for 12 *P. melisellensis* populations using Bayesian GEA methods, and their overlap with 46 loci identified as putatively under selection in Pod Mrčaru and Pod Kopište populations (marked in square brackets). Overlap of loci found significant across different methods is noted in the last column (*one locus shared across 2 environmental variables). Variable abbreviations are defined in Table 2.

Variable	Bayenv2	Baypass	BayeScEnv	GEA overlap
Longitude	49 [-]	227 [-]	77 [-]	-
Latitude	30 [-]	1155 [-]	- [-]	-
Area	27 [-]	414 [-]	141 [-]	-
Altitude	34 [-]	274 [-]	207 [-]	-
DistanceToLand	111 [-]	187 [-]	119 [-]	-
DistanceToLargeIsland	125 [-]	341 [-]	167 [-]	5*
DepthToCoastMax	76 [-]	286 [-]	179 [-]	-
TemperatureMean	27 [-]	430 [-]	- [-]	-
TemperatureMin	26 [-]	566 [-]	2 [-]	-
TemperatureMax	84 [-]	99 [-]	180 [-]	-
TemperatureRange	29 [-]	534 [1]	64 [-]	-
PrecipitationMean	31 [-]	408 [-]	136 [-]	-
PrecipitationMin	31 [-]	633 [-]	57 [-]	-
PrecipitationMax	39 [-]	367 [-]	143 [-]	-
PrecipitationRange	47 [-]	167 [-]	150 [-]	-
SolarRadiationMean	14 [-]	438 [-]	116 [-]	-
SolarRadiationMin	8 [-]	2518 [3]	- [-]	-
SolarRadiationMax	10 [-]	1123 [-]	34 [-]	-
SolarRadiationRange	22 [-]	587 [-]	63 [-]	-
WindSpeedMean	31 [-]	631 [-]	52 [-]	1*
WindSpeedMin	33 [-]	299 [-]	93 [-]	-
WindSpeedMax	31 [-]	502 [-]	105 [-]	-
WindSpeedRange	29 [-]	1828 [2]	- [-]	-

In Baypass core model analysis, 414 loci were further identified as putatively under selection in all *P. melisellensis* populations – 222 of them under directional selection, 192 under balancing selection (Supplementary material, Figure S20).

No significant overlap was detected between loci found significant in Bayesian GEA analyses of *P. siculus* and *P. melisellensis* populations, or between loci found significant in GEA analyses of *P. melisellensis* populations and those identified as putatively under selection in Pod Mrčaru and Pod Kopište populations.

4.3.4 Signatures of genomic adaptation

Out of 46 candidate loci identified as putatively under selection in Pod Mrčaru and Pod Kopište *P. siculus* populations using several different genome scan methods, 17 were also associated with divergent phenotypic traits in LFMM genotype-phenotype analysis of these two focal populations (Figure 26). Moreover, 6 out of 46 putative candidates for selection in Pod Mrčaru and Pod Kopište were also identified as under selection across all investigated *P. siculus* populations based on Baypass XtX statistics (Figure 26). Additionally, 4 unique loci were found to show both signs of selection in two focal populations and significant association with various environmental variables in Bayenv and BayPass genotype-environment analysis of all sampled *P.siculus* populations.

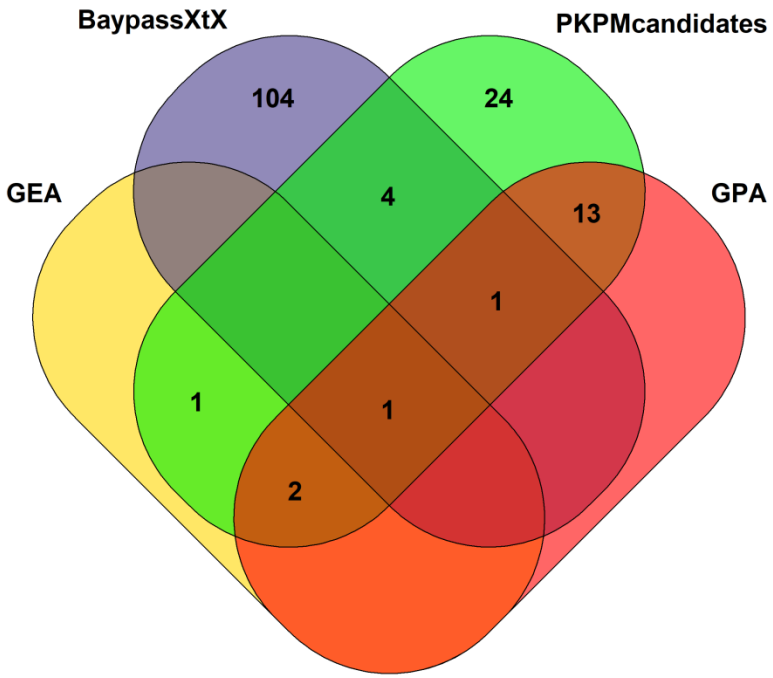


Figure 26 Overview of putatively adaptive loci in Pod Mrčaru and Pod Kopište *P. siculus* populations found in genome scans for selection (PKPMcandidates) and genotype-phenotype association analysis (GPA) on two focal populations, as well as the genome scan for selection (BaypassXtX) and genotype-environment analysis (GEA) on all sampled *P. siculus* populations.

5. DISCUSSION

5.1 Variation in parental and offspring phenotype

Patterns of phenotypic variability in wild populations may vary across spatial and temporal scales due to plastic responses towards temporary ecological changes and/or due to fluctuations in the strength of selection acting on genomic variation underlying adaptive phenotypes (Hendry, 2017; Michel et al., 2014; Siepielski et al., 2009). Nonetheless, the observed phenotypic differentiation between adult *P. siculus* individuals sampled on Pod Kopište and Pod Mrčaru and used as parental F0 generation in the crossing experiment closely followed the pattern described by Herrel et al. (2008) more than a decade ago. Both male and female *P. siculus* lizards from Pod Mrčaru had larger bodies and bigger heads that individuals sampled on Pod Kopište, a trend evident across all analysed phenotypic traits. The persistence of this adaptive phenotypic response over such long period of time suggests continued influence of divergent selection, which drives the populations towards different ecological optimums (Hendry, 2017). However, the same pattern of phenotypic divergence was not found in sub-adult F1 individuals raised in the common garden, with almost no significant difference detected between analysed traits in male and female offspring from Pod Mrčaru and Pod Kopište. These results are contrary to what was found in the wild, where the differences in head size between juveniles caught on the two islands mirrored that of their parents, with Pod Mrčaru individuals having significantly longer and wider heads than those on Pod Kopište (Herrel et al., 2008). The discrepancy between phenotypic variation observed in the individuals sampled from their natural habitat and those raised in the common garden could potentially be attributed to partial loss of phenotypic variability in offspring due to shared rearing environment and diet regime. This may indicate that differences between populations themselves are not heritable, which would also point towards at least partially plastic response to change in diet in wild lizards, as was previously suggested by Vervust et al. (2010).

On the other hand, the observed disparity could similarly be due to the relatively small number of F1 individuals obtained per population and sex, and the differences in age-at-measurement between parental and offspring lizards in this experiment. Namely, while all parents were sampled and phenotyped as full adults, offspring were still sub-adults and had smaller body and head sizes than parental generations at the time of their phenotypisation. The observed phenotypic differences between parents and offspring might thus also be due to

different growth rates of individuals in captivity, as all head measures were standardized by body length before further analyses. Head size in particular is believed to be important for sexual selection in lizards – larger bodied males with bigger heads dominate in male-male competitions and may have higher reproductive success (Herrel et al., 2010; Lailvaux et al., 2004; Scharf and Meiri, 2013). The similarity of phenotypic traits in sub-adults, and their variation in adult male and female lizards, could also indicate that analysed traits are to some extent sexually selected or expressed in the process of sexual maturation in *P. siculus* individuals from Pod Mrčaru and Pod Kopište islands.

5.2 Heritability of putatively adaptive phenotypic traits

Despite the lack of clear trends that would point towards differences between populations being heritable themselves, high within-population heritability estimates were found for all traits connected to head size in *P. siculus* individuals from Pod Mrčaru and Pod Kopište. This certainly suggests that phenotypic variation among investigated individuals is to a considerable extent due to variation in the heritable additive genetic component. Basic ANOVA and PCA analyses were performed by dividing the phenotyped individuals into 8 distinct groups (four cross types, and two sexes), which might have resulted in insufficient number of observations per group and high within group variability. On the other hand, animal models were based on joint analysis of all experimental individuals, and influence of additive genetic component on variation in traits of interest was detected after accounting for mean phenotypic difference between the groups. Although most other studies conducted so far focused primarily on coloration variation, behaviour or physiological performance (Ljungström et al., 2016; Logan et al., 2018; Martins et al., 2019; Rankin et al., 2016), high heritability estimates have been previously recorded for both head size and shape, as well as other morphological body traits across several lizard species. For instance, Sacchi et al. (2016) estimated narrow-sense heritability for head size in common wall lizard (*Podarcis muralis*) at $h^2 = 0.53$, using a REML animal model approach and cephalic landmark configuration centroid size as a proxy for head size. Imhoff et al. (2018) likewise employed centroid size as proxy for head size and a linear regression approach designed specifically for shape measurements, but obtained implausibly high values of heritability for head size ($h^2 > 1$) in Argentine black and white tegu (*Salvator merianae*). Moreover, such estimates can rarely be connected to specific adaptive processes in the wild directly, as is the case here. Putatively adaptive thermoregulatory traits, developed under different environmental

conditions, for instance, show low heritability values across multiple lizard species (Logan et al., 2018; Martins et al., 2019).

As an indicator of overall body size, length to hip specifically might be important for selection (Malenfant et al., 2018; Noordwijk et al., 1988). Indeed high heritability ($h^2 > 0.5$) of snout-vent length (comparable to length to hip analysed here) was previously recorded in Australian rainforest sunskink (Martins et al., 2019) and *Anolis* lizard species (Calsbeek and Smith, 2007). However, relatively low heritability estimates for body size obtained in this study suggest more plastic response in Pod Mrčaru and Pod Kopište populations, developed under environmental or parental influence. High influence of maternal genetic component on the variability in snout-vent length was, for example, reported in Eastern water skink lizard (*Eulamprus quoyii*) (Noble et al., 2014). Similarly, bite force is expected to be an important performance trait because it's directly linked to variation in lizards diet (Herrel et al., 2004; Taverne et al., 2020; Verwaijen et al., 2002), as well as the inter- and intra-species competition which affects resource acquisition and mating preference (Donihue et al., 2016; Herrel et al., 2007; Lailvaux et al., 2012; Lappin and Husak, 2005). However, a thorough search of the relevant literature reviled no studies estimated heritability of bite force in lizards, or even vertebrates thus far. Extremely low and sometimes negative heritability values were recorded for bite force in this analysis. In general, heritability of performance traits important for fitness, such as bite force, is expected to be low due to the depletion of additive genetic variation that results from selection (Hoffmann et al., 2016; Mousseau and Roff, 1987). However, this does not appear to be the case here as Pod Mrčaru population was established from Pod Kopište quite recently and notably diverged in bite force in relatively short time. Therefore, if additive genetic variance did underlie phenotypic differentiation in bite force it would still be detected in Pod Kopište individuals. Other studies similarly suggested that low heritability found in fitness-related traits might reflect disproportionately large contribution of environmental or non-additive genetic factors to their variation, rather than a reduction in underlying additive variance (Kruuk et al., 2000; Teplitsky et al., 2009). Results presented here thus imply important role of environmental plasticity in bite force variation across natural lizard populations, as has been suggested previously by Irschick and Meyers (2007). Although high heritability ($h^2 > 0.5$) has been previously recorded for tail length in both scincid and larcertid lizard species (Martins et al., 2019; Sorci et al., 1995), in this experiment tail varied wildly in length across different individuals, was often discarded during lizard handling, and regenerated at different rates. Because low estimates of additive

genetic component and heritability were quite expected for such randomly varied trait, tail length was treated as a "control case" in comparisons across different analysis used in this study.

It is apparent that full-sib ANOVA, as well as parent-offspring regression and – to some extent – ASSOC analyses, struggled with relatively low number of samples obtained in this experiment, which violated basic assumptions about balanced design, normality and homoscedasticity of the dataset that are expected in regular linear models. Theoretically, negative heritability values or values greater than one are statistically impossible because under the additive genetic variance definition, heritability is expressed as a proportion in which the numerator is contained in the denominator. However, negative values can appear in experimental studies if the model assumptions used to estimate heritability were not met and if some of the variation in the offspring phenotype is due to non-additive (gene-gene interactions at different levels), or non-genetic (environmental) variation (Gill and Jensen, 1968; Huneman and Walsh, 2017; Steinsaltz et al., 2020). Similarly, heritability values greater than one can be obtained when the correlation among relatives included in the study is greater than expected, mostly due to inbreeding or dominance effect (Verma and Agarwal, 2010). It can be concluded that the dataset obtained in this study does not meet the requirements needed for a thorough analysis of variance among full-siblings. The ANOVA-obtained estimates of heritability are therefore not valid, and cannot be considered significant in the interpretation of genetic and plastic responses governing differentiation in the investigated phenotypic traits.

Animal models, on the other hand, generally provide more robust statistical support for inferring heritability patterns in wild animal populations, because they do not require balanced datasets and invoke fewer assumptions about patterns of selection and/or inbreeding in analysed individuals. Another advantage of animal models over traditional approaches is the simultaneous estimation of both genetic and environmental influences governing variation in phenotypic characteristics (Ellegren and Sheldon, 2008; Kruuk, 2004; Wilson et al., 2010). This allows for the estimation of selective pressure that is unbiased by variability in factors such as sex, age, and differences in habitat or parental populations. Not accounting for sex-specific trait variability, for example, can lead to partial partitioning of the variability components, because the same type of genotype-environment correlation is assumed for each sex or population, when this is often not the case (de Villemereuil et al., 2018a; Kruuk et al., 2008; Wolak et al., 2015). In fact, sexes can often respond differently to changes in selection

pressure, leading to divergent patterns of adaptation in the nature (Singh and Punzalan, 2018; Svensson et al., 2018). However, including sex as a fixed effect in the models explored here, lowered the overall model fit, and decreased estimates of additive genetic variance component and heritability. This is surprising, but could be due to bias introduced by previously mentioned difference in age-at-measurement, and consequently body size and sexual dimorphism of the traits between analysed parental and offspring generations.

Diverse genotype-environment association patterns are also likely to develop among individuals raised in different environmental conditions (Quéméré et al., 2018; Wilson et al., 2010). Modelling the experimental year as fixed effect allowed accounting for difference between F0 individuals which spent year in Zoo or were paired after sampling in the field, differences in rearing environments experienced by parents in the wild and offspring hatched in two successive years (2017 and 2018 F1 cohort) as well as, in part, the difference in their body sizes during phenotypisation. This, in turn, decreased the estimates of residual variance component and increased the overall heritability projections, as anticipated. Similarly, different genotype associations can be expected in potentially genetically diverging populations (Muff et al., 2019). Accounting for population effect in addition to experimental year and/or sex in this study, further increased the overall heritability projections, suggesting lingering between-population differences based on previously induced environmental response. Because of the variation in genomic background and different genotype-environment interactions, heritability estimates are population and environment specific and cannot be extrapolated from one population or one environment to another. Fitting population as fixed effect in animal models, thus allowing the populations to differ in phenotypic mean, is a simple way to model permanent environmental differences among analysed groups (Hadfield et al., 2010). However, such approach might not be appropriate for putatively genetically differentiated populations, because all analysed groups are assumed to harbour the same amount of additive variance. This could be further addressed by implementation of animal models with genetic groups (Muff et al., 2019; Wolak and Reid, 2017), which may reduce potential bias in parameter estimation and allow assessment of difference between the amount of additive genetic variation underlying traits of interests in Pod Mrčaru and Pod Kopište populations. Due to the limitations of the analysed pedigree it was impossible to estimate the influence of other environmental and/or non-additive factors (i.e. dominance, maternal or permanent environment effects), which might also contribute to phenotypic variation in analysed traits of interest.

The results presented here elucidate basic patterns of genetic variance underlying the adaptive phenotypic response in Pod Mrčaru *P. siculus* population. High heritability of phenotypic traits connected to head size and shape in *P. siculus* lizards suggests the existence of large additive genetic component, which would allow these traits to evolve under selection pressure. However, relatively large standard errors and/or confidence intervals point towards low precision of obtained estimates, and a thorough QTL and GWAS analysis on bigger dataset should be employed in the future in order to evaluate the in-depth genetic architecture of adaptive traits studied here.

5.3 Genomic diversity in wild populations

The establishment of a new population into an isolated environment is often expected to result in pronounced genomic divergence, making islands some of the most famous examples of remarkable biodiversity we see today (Warren et al., 2015). However, because such isolated populations are usually founded by only few individuals, they carry only a random subset of alleles present in the source population, which leads to a special case of bottleneck, called founder effect (Mayr, 1942). Founder effect further enhances genetic drift that, coupled with restricted gene flow, leads to rapid loss of genetic diversity (Frankham, 1997; Kolbe et al., 2012) which can hamper populations ability to adapt and persist in the new environment (Agashe et al., 2011; Hughes et al., 2008; Reed and Frankham, 2003). In this context, basic population genomics diversity indices help describe the amount of standing genetic variation in investigated populations and provide an insight into their evolutionary potential and viability (Barrett and Schluter, 2008; Lai et al., 2019).

Variable levels of heterozygosity and allelic richness were recorded across species and populations investigated in this study. *P. siculus* showed higher genomic diversity than *P. melisellensis*, which is in accordance with differences in biology and ecology between the two species. Namely, while *P. melisellensis* is considered to be an autochthonous species, *P. siculus* is a relatively new invader in this area (Gorman et al., 1975; Podnar et al., 2005, 2004; Radovanović, 1956). Studies suggest that increased genetic diversity in founder populations increases colonization success (Crawford and Whitney, 2010; Forsman, 2014; Szűcs et al., 2017), and high levels of standing genomic variation may facilitate *P. siculus* successful adaption to new habitats along its expansion range. These results were further corroborated by AMOVA analysis which likewise showed that high amount of genomic variation exists within investigated *P. siculus* individuals. Mainland populations are similarly expected to

harbour more genetic diversity than insular populations, because they are not as strongly effected by genetic drift and restricted gene flow (Bichet et al., 2015; Frankham, 1997; Wang et al., 2014; White and Searle, 2007). Indeed, *P. siculus* populations from the islands in the 'Split' group showed higher diversity than lizards from Lastovo archipelago, potentially due to sporadic gene flow from the nearby coastline or more recent colonisation of this area. Moderate genomic variability was additionaly found in most *P. siculus* populations from 'Lastovo' group, which may be due to the high geographic proximity of islands in this region facilitating gene flow or their ecological similarity. Similar pattern was also seen in *P. melisellensis* populations inhabiting Vis archipelago, and may be driven by the fact that those islands were connected by land until relatively recently in their geological past (Podnar et al., 2004). On the other hand, few populations did appear to be significantly affected by low genomic diversity, which could affect their future viability – specifically *P. siculus* from Bijelac, and *P. melisellensis* populations inhabiting Mali Barjak, as well as the isolated volcanic islands (Brusnik, Jabuka, Kamik) and islands near Lastovo (Veli Tajan and Glavat)., However, diversity was not strongly related to habitat area, and several island populations seem to harbour substantial amount of standing genomic variation despite putative isolation and small population size.

Similar trend could be also observed in Pod Mrčaru *P. siculus* population. While the initial founder effect was expected to result in significant loss of genomic variability, *P. siculus* population from Pod Mrčaru showed only slightly lower diversity than was recorded on Pod Kopište (Ho = 0.023, Ar = 1.076 vs. Ho = 0.025, Ar = 1.086, respectively). In fact, the amount of standing genomic variation seen in Pod Mrčaru was higher than at least one other *P. siculus* population in Lastovo archipelago (Bijelac). Relatively high genomic diversity observed on Pod Mrčaru contradicts the theory presented by Vervust et al. (2008) who speculated that severe bottleneck and consequent decrease in genetic variability were the main drives behind some of the phenotypic changes observed in this population. Instead, higher than expected diversity observed in this study points toward significant influence of more subtle evolutionary mechanisms, such as the effective purging of deleterious alleles, which is known to reduce inbreeding depression in small isolated populations (Facon et al., 2011; Grossen et al., 2020; Robinson et al., 2018); associative overdominance that may result in increased heterozygosity at neutral loci due to their linkage to loci under selection (Pamilo and Paælsson, 1998; Schou et al., 2017); or high population growth rates, which have a

potential to limit the amount of genomic variation lost during a bottleneck (Allendorf, 1986; Fuller et al., 2020; Hundertmark and van Daele, 2010; Murphy et al., 2015).

For example, Fuller et al. (2020) found that population of white-tailed deer (*Odocoileus virginianus*) introduced to Anticosti Island in Canada in the late 19th century shows higher heterozygosity and lower inbreeding than the ancestral and other comparable mainland populations. This fact was attributed to the large number of founders (>200) and rapid population growth after the introduction. Although the number of founders on Pod Mrčaru Island was considerably smaller and consisted of only ten *P. siculus* individuals (Nevo et al., 1972), both field observations and prior surveys suggest that large *P. siculus* population exists on Pod Mrčaru today. Namely, both Herrel et al. (2008) and Vervust et al. (2009) observed much higher density of *P. siculus* individuals on Pod Mrčaru than on Pod Kopište. If *P. siculus* on Pod Mrčaru did experience rapid population growth after the initial bottleneck, this could have had a positive effect on the amount of genomic diversity retained in the population (Allendorf, 1986; Kirkpatrick and Jarne, 2000; Murphy et al., 2015). Similarly, associative overdominance – which arises as the product of linkage disequilibrium between neutral loci and loci under selection – can promote the maintenance of neutral genetic variation in small populations experiencing bottleneck (Frydenberg, 1963; Pamilo and Paælsson, 1998). Moreover, in the presence of multiple recessive deleterious alleles on different loci, associative overdominance may result in increased fitness advantage of the heterozygotes, which could further explain the apparent lack of inbreeding depression in investigated populations (Charlesworth and Willis, 2009; Schou et al., 2017; Wetzel et al., 2012; Zhao and Charlesworth, 2016).

5.4 Patterns of genomic differentiation among wild populations

Distinct population structure, characterised by several divergent genomic clusters, was found for both species investigated in this study. This trend was consistent across different analytical methods and highlighted the separation of *P. siculus* populations into two highly differentiated groups. Similar demographic patterns were reported from mitochondrial markers, and are in accordance with phylogeography and invasive migration of *P. siculus* across Adriatic islands of the coast of Croatia (Gorman et al., 1975; Podnar et al., 2005; Radovanović, 1956). For instance, two distinct *P. siculus* haploclades were previously described along the area of Adriatic coast investigated in this study based on variation in mitochondrial cytochrome *b* and 12S and 16S rRNA sequences (Podnar et al., 2005). The

repeated phylogenetic analysis on number of *P. siculus* populations (Taverne et al. (2020); Supplementary material, Figure S21) corroborated those results and genomic data further supported the observed divergence. In particular, populations from 'Lastovo' group detected in this study were equivalent to those belonging to *Sušac* clade, while 'Split' group broadly corresponded to the *Adria* haploclade identified by Podnar et al. (2005). Genomic analyses, however, also pointed towards the existence of finer population structuring within those two groups. Islands Pijavica and Kluda, for instance, clustered separately and showed lower differentiation from 'Split' than other 'Lastovo' populations, indicating potential introgression of 'Split' genomic variants due to their geographical proximity to the coast. Additionally, moderate levels of divergence were detected among mid-Adriatic islands situated at the north range of the studied area, where at least three additional genomic clusters were identified. However, to what extent different colonisations histories, genetic drift and adaptive processes influence this observed divergence across a relatively small geographic area is still unclear.

On the other hand, *P. melisellensis* exhibited strong genomic differentiation between almost all analysed populations. In a study similar to that conducted on *P. siculus*, Podnar et al. (2004) reported on "unresolved trichotomy" of three distinct *P. melisellensis* haploclades in the investigated area of Adriatic coast: *fiumana* clade, connected to mainland and nearby islands, *Lastovo* clade, containing islands inhabited by *P. melisellensis* in the Lastovo archipelago; and finally *melisellensis* clade, comprised of populations on the islands in Vis archipelago, including the three volcanic islands analysed here. While the repeated phylogenetic analysis generally corroborated those results, no clear separation between two populations sampled from islets near Lastovo (Glavat and Veli Tajan) and mainland population of Sinj was recorded (Taverne et al. (2020); Supplementary material, Figure S21). In particular mitochondrial DNA from northern *fiumana* clade was found on Veli Tajan, while separation between *fiumana* clade and population from Glavat – which was previously described as *Lastovo* clade (Podnar et al., 2004) – was not well supported. Genomic approach likewise pointed towards closer relatedness of Glavat and Veli Tajan to mainland population of Sinj than to Vis group of populations, but also showed distinct separation of *P. melisellensis* on Veli Tajan. Similar pattern of divergence in population from Veli Tajan was previously described based on morphometric data (Clover, 1979; Thorpe, 1980), and could be a sign of relatively recent anthropogenic introduction of other genomic backgrounds. Population genomic analyses further highlighted clear divergence of populations inhabiting

volcanic islands (Brusnik, Jabuka and Kamik) from others in Vis archipelago, but also pointed towards strong differentiation among populations within identified genomic clusters themselves. AMOVA analysis for instance, showed that variation among populations within genomic clusters was much higher in *P. melisellensis* (17.87%) than *P. siculus* (7.2%), even though smaller number of clusters was detected across *P. melisellensis* populations in general. Extremely high F_{ST} values (up to 0.93) were also recorded for some pairs of *P. melisellensis* populations, which even exceed those observed between populations of two different species investigated here. This pattern, coupled with high phenotypic divergence among some of these populations (Clover, 1979; Gorman et al., 1975; Podnar et al., 2004; unpublished data) and diverse environments on the islands, is indicative of on-going speciation among analysed *P. melisellensis* populations, which is further expected considering the long history of this species in the investigated area (Gorman et al., 1975; Podnar et al., 2004).

Genomic analysis of population differentiation among sampled populations thus both confirmed and extended the previous findings, revealing the existence of much more intricate population structure in *P. siculus* and *P. melisellensis* along the Adriatic coast than could be described using phylogenetic markers. Results presented here demonstrate the high resolution that can be obtained by employing genomic tools for the in-depth study of demographic processes in nature, especially in cases where divergence is occurring on contemporary timescales (Allendorf, 2017; Fumagalli et al., 2013; McCormack et al., 2013). Indeed, while no difference was found in previous mitochondrial DNA analysis of Pod Mrčaru and Pod Kopište *P. siculus* individuals (Herrel et al., 2008), this study demonstrates clear genome-wide divergence between these two populations. Although the genomic assignment of Pod Mrčaru and Pod Kopište *P. siculus* populations to the same cluster in *fastStructure* was expected due to the model assumptions about global ancestry parameters (Lawson et al., 2018; Raj et al., 2014), modest but significant differentiation between two populations was detected using both the population fixation index and PCA analysis of genomic variance. Moreover, this differentiation is on par with the levels of genomic divergence observed in the surrounding populations – for example, differentiation between Pod Kopište and Pod Mrčaru (F_{ST} = 0.04) is only twice lower than differentiation found between Pod Kopište and Kopište, the next closest island inhabited by *P.siculus* (F_{ST} = 0.08). These values are also comparable to those obtained in other studies which attempted to quantify recent genomic divergence between ancestral and introduced populations, e.g. in white-tailed deer (Fuller et al., 2020),

giant threespine stickleback (Marques et al., 2018), and green anole lizard (Tamate et al., 2017).

Interestingly, *P. melisellensis* population from Veli Barjak appeared to exhibit signatures of potential genomic introgression from *P. siculus* species. This trend was discernible across all methods employed to test genomic diversity and differentiation among wild populations in this study – *P. melisellensis* individuals from Veli Barjak showed lower differentiation from *P. siculus* than was recorded for other investigated *P. melisellensis* populations, clustered closer to *P. siculus* in the PCA decomposition of genomic variance between two species, and displayed a considerable degree of admixture with *P. siculus* in population assignment analyses based on individual ancestry. Additionally, highest estimates of heterozygosity and allelic richness in all analysed *P. melisellensis* populations were recorded precisely on Veli Barjak, implying a potential increase in genomic diversity due to introduction of foreign genomic variants (Grant and Grant, 2019; Hedrick, 2013; Sagonas et al., 2019). Although *P. siculus* is known to hybridise with other *Podarcis* species (Capula, 2002, 1993), hybridization between *P. siculus* and *P. melisellensis* was not recorded in nature thus far. Given that only one analysed population in this study showed any signs of admixture between the two investigated species, it further appears special ecological circumstances are needed for introgression between *P. siculus* and *P. melisellensis* to occur. Introgression between these two species was previously suggested to exist on Pod Mrčaru by Gorman et al. (1975), who performed a genetic survey of Adriatic lizards at the time of the experimental introduction of *P. siculus* on Pod Mrčaru Island in 1971, then inhabited by *P. melisellensis*. Based on variation in allozyme markers Gorman et al. (1975) speculated that *P. melisellensis* population on Pod Mrčaru was experiencing introgression due to occasional immigration of individuals from *P. siculus* population on Pod Kopište. Though it can be tempting to hypothesise that putative genomic introgression between *P. siculus* and *P. melisellensis* played an important role in the early days of *P. siculus* establishment on Pod Mrčaru Island, the results obtained in this study show no evidence of such process occurring in the two focal populations investigated in this study.

5.5 Signatures of selection in Pod Mrčaru and Pod Kopište populations

Genomic differentiation between isolated populations could be the product of reduced gene flow and enhanced drift, along with selection acting on adaptive genomic variants. Over the years multiple genome scans methods have been developed to detect changes in allele

frequency distributions induced by selection, mainly by looking at loci-specific F_{ST} values and how they deviate from the assumed demographic equilibrium (Excoffier et al., 2009; Foll and Gaggiotti, 2008; Whitlock and Lotterhos, 2015). Namely, because neutral processes such as drift or gene flow affect all loci across the genome equally, all neutral loci are assumed to have approximately the same F_{ST}. On the other hand, selection will impact only specific adaptive or linked loci, which are expected to show significantly higher or lower F_{ST} values than neutral – suggesting evidence of directional or balancing selection, respectively (Lotterhos and Whitlock, 2014). The underlying model used to simulate neutrality will thus have a large impact on identification of loci under selection, and deviations from assumed parameters may result in increased number of false positives (François et al., 2016; Lotterhos and Whitlock, 2014; Narum and Hess, 2011).

To account for potential departures from underlying models, multiple methods based on different algorithms were used to identify candidate loci for selection in Pod Mrčaru and Pod Kopište *P. siculus* populations. For instance, Bayescan software used in this study is based on multinomial Dirichlet distribution which assumes that samples have diverged independently from a common ancestor (Foll and Gaggiotti, 2008). Although Bayescan is known to be robust towards both Type I (false positives) and Type II (false negatives) errors across several different demographic scenarios (Narum and Hess, 2011; Pérez-Figueroa et al., 2010), it also assumes that investigated populations are evolutionary independent. The stringent parameterization and presence of admixed individuals might have resulted in increase of false negatives and, consequently, only one loci identified as under selection using Bayescan in this study (Luu et al., 2017). On the other hand, non-hierarchical finite island model was used as a null distribution to test for significance in software Arlequin (Excoffier and Lischer, 2010). Arlequin is known to be less conservative than Bayescan (Narum and Hess, 2011), and has indeed identified more candidate loci for selection in the two populations investigated here. Those could signify false positives, however Type I errors in Arlequin were shown to be of most concern in the presence of strong hierarchical structure (Narum and Hess, 2011) which is not the case here. In addition, the majority of loci detected by Arlequin were identified as candidates for selection in PCAdapt as well. In contrast to the two abovementioned model-based genome scan methods, PCAdapt software does not assume specific demographic history, but uses estimates of covariance among individuals to account for hierarchical structure in the test statistic, thus allowing for evolutionary non-independence among samples (Luu et al., 2017). Moreover, simulations have shown PCAdapt is less prone

to Type 2 errors than Bayescan, especially in scenarios of population divergence and range expansions with admixed individuals (Luu et al., 2017). Lastly, although the PCA analysis of allelic frequencies as implemented in R package *adegenet* (Jombart, 2008) is not a standard approach to determine loci putatively under selection, its application allowed verifying that loci detected by genome scan methods are indeed among those that contributed the most to genomic differentiation between investigated populations.

Across the different methods used in this study, 46 loci were identified as putative candidates for selection in Pod Mrčaru and Pod Kopište *P. siculus* populations. These loci represented 1.9% of all SNPs in the analysed dataset, which is further in accordance to 1-5% range of overlap shown to be standard in comparisons among different genome scan methods (de Villemereuil et al., 2014). However, some detection bias could still be expected due to complex demographic history of the investigated system. In particular, none of the utilised methods was specifically designed to account for non-equilibrium conditions involved in the transplant experiment and severe bottleneck that Pod Mrčaru population experienced at the time of its introduction (Excoffier et al., 2009; Foll and Gaggiotti, 2008). Although addressing this concern was outside the scope of this research, simulations based on null expectations tailored to the specific history of this system should be further employed in order to validate candidate loci identified in this study. It is also important to notice that GBS protocol entails reduction of the whole genome into many short, randomly distributed DNA sequences (Elshire et al., 2011) and thus many other genomic variants important for adaptive differentiation between focal populations may have been missed during library preparations.

5.6 Genotype-phenotype interaction in two focal populations

In contrast to genome scans methods that rely on loci-specific F_{ST} deviation from equilibrium to detect outliers putatively under selection, genotype-phenotype-environment associations analyses are based on correlation between allele frequencies and variation in phenotypic or environmental factors (de Villemereuil et al., 2014; Lotterhos and Whitlock, 2014). Such methods are more likely to detect subtle changes in allele frequencies based on spatially variable factors and are able to detect divergent selection even if does not produce strong differentiation among populations (Flanagan et al., 2018; François et al., 2016; Rellstab et al., 2015). When looking for basis of genomic adaptation in the wild, it is thus frequently recommended to augment genome scans with genotype-phenotype or genotype-environment association methods.

Indeed, LFMM analysis of genotype-phenotype associations in Pod Mrčaru and Pod Kopište individuals detected a notable number of significant SNPs across almost all analysed phenotypic traits. LFMM was shown to perform as well as the standard genome-wide association (GWA) algorithms in both simulated and experimental studies (e.g. well-known linear mixed model implemented in GEMMA software, see Frichot et al., 2013; Jacobs et al., 2019), and has over the last couple of years become a popular tool to asses genotype-phenotype associations in the wild. For example, LFMM was recently used to identify loci underlying colour polymorphism in European fire salamander (Burgon et al., 2020), plateless phenotype in the threespine stickleback (Mazzarella et al., 2016), genomic background of morphological differences among lake trout ecotypes (Perreault-Payette et al., 2017), and insular and mainland populations of white-tailed deer (Fuller et al., 2020). The LFMM results presented in this study are in accordance with signatures of polygenic architecture and adaptive nature of investigated phenotypic traits, as well as the observations from previous studies that focused on differentiation between pod Mrčaru and Pod Kopište *P. siculus* populations. For instance, Herrel et al. (2008) notably described high evolutionary rate of divergence (up to 8,593 Darwins or 0.049 Haldanes) in head height, length and width between wild Pod Mrčaru and Pod Kopište *P. siculus* individuals. Those traits, along with snout length and lower jaw lever and outlever, modulate overall head size and shape and are directly connected to individuals bite force – lizards with larger heads tend to have stronger bite force, which is further associated with the ability to include tougher food items, such as plants, into their diet (Herrel et al., 2004; Taverne et al., 2020; Verwaijen et al., 2002). Large number of loci associated with various head size traits in LFMM genotype-phenotype analysis of both female and male individuals thus also reaffirmed the results obtained from the crossing experiment in the common garden and pointed toward large additive component underlying phenotypic variance in those traits. Low overlap among loci detected in the analyses of female and male individuals, and higher number of loci associated with phenotypic traits found in male individuals in general, further suggested stronger selective pressures acting on analysed head shape traits in males than females (Fuller et al., 2020). However, genotype-phenotype association patterns detected using LFMM may be sensitive to physical linkage among investigated loci (Caye et al., 2019). Specifically, high LD between trait-associated and non-associated loci may suggest that detected SNPs are not the optimal ones for tagging causal variants. As the estimation of genome-wide LD was not accounted for in this analysis, more detailed association studies might be necessary to corroborate the obtained results.

While comparison of phenotypic (P_{ST}) and genetic (F_{ST}) differentiation is not a strict genotype-phenotype association method, it allows evaluating the relative contribution of selective and neutral processes to phenotypic divergence between populations of interest (Brommer, 2011; Leinonen et al., 2013). Interestingly, traits that showed highest phenotypic differentiation in comparison with genomic divergence across both sexes were those closely connected to body size and locomotor function (i.e. snout-vent and limb length), while no such prominent difference between genomic and phenotypic divergence was found for head shape traits. Apparently high influence of directional selection on phenotypic differentiation in body size is contrary to results obtained from the crossing experiment that pointed towards more plastic response and low heritability of overall body size. The approximation of Q_{ST} by P_{ST} is in general considered highly dependent on underlying assumptions regarding the magnitude of environmental effects on within and among population variance (Brommer, 2011; Leinonen et al., 2013). In particular, P_{ST} estimation is expected to be problematic for traits that show low estimates of heritability because of their greater sensitivity towards changes in environmental factors (Brommer, 2011). Similar difference between results obtained from P_{ST} estimation in the wild and crossing experiments in the common garden was, for instance, also reported for the degree of melanism differentiation in common frog, *Rana temporaria* (Alho et al., 2010). Differentiation in limb length is, on the other hand, a rather famous example of phenotypic adaptation to distinct perching substrates in Anole lizards – for example thin branches in twig and crown, or flat surfaces in trunk and ground dwelling ectomorphs (Langerhans et al., 2006; Losos, 1990). Limb length was also previously identified as important for divergence between Pod Mrčaru and Pod Kopište *P. siculus* populations by Vervust et al. (2007), who attributed the change to lack of predation pressure on Pod Mrčaru. Conversely, differences in limb length between the two populations may also reflect behavioural and physiological changes that stem from underlying shift in diet in Pod Mrčaru *P. siculus* lizards, i.e. change in foraging style from active pursuit of mobile prey to browsing (Herrel et al., 2008). However, more detailed quantitative genetic studies should be employed to further distinguish between relative influences of selection and drift driving the phenotypic divergence in these traits.

5.7 Genotype-environment associations in wild *Podarcis* populations

Loci that correlate with environmental variables are presumed to fluctuate in allelic frequencies in response to selective pressures, and are thus highly indicative of adaptive

evolution at the scale of population (Coop et al., 2010; Forester et al., 2018; Rellstab et al., 2015). Environmental factors affecting fitness of individuals within populations can act selectively on the genes underlying numerous cellular and physiological processes, which further underlie adaptation to other environmental distributions (Hoban et al., 2016; Pardo-Diaz et al., 2015). Loci pinpointed in GEA analysis are not thus only of importance for the environmental factor with which they correlate, but for adaptive response of wild populations in general. Applying the GEA analysis in multi-population two species framework further facilitates research of evolutionary parallelism and adaptive convergence (Hohenlohe et al., 2010; Prates et al., 2018; Thorpe et al., 2015; Wood et al., 2005).

Different signatures of local adaptation were, however, recorded across *P. melisellensis* and *P. siculus* populations investigated in this study. The loci that were important for selection in Pod Mrčaru and Pod Kopište or for environmental associations across all investigated *P. siculus* populations did not appear to be adaptive in *P. melisellensis* lizards. Indeed, in comparison to *P. siculus*, *P. melisellensis* showed relatively few loci in association with changes in environmental factors across investigated sites. Apart from one locus associated with mean wind speed, only distance to nearest large island showed any significant interaction with genomic variation among *P. melisellensis* populations across all three Bayesian GEA analyses. These results point toward extremely strong influence of geographic isolation on genomic differentiation in *P. melisellensis* – an observation that was further supported by RDA analysis which showed geography had higher effect than ecology on the distribution of genomic variation among *P. melisellensis* populations. In fact, when looking at RDA results both geography and ecology explained notably less genomic variation in *P. melisellensis* than in *P. siculus*. *Podarcis melisellensis* has a long evolutionary history of inhabiting limited environments within the investigated area, and shows colonisation pattern which is further suggestive of local evolutionary radiation (Podnar et al., 2004). Considered in conjunction with the results obtained from the analysis of genomic diversity, the trend observed in GEA study may be underlined by joint influence of drift and purifying selection (Charlesworth et al., 1993; Frankham, 1997; Robinson et al., 2018), which would result in investigated *P. melisellensis* populations having lower amount of adaptive variation left for selection to act upon (Reed and Frankham, 2003). However, the relatively low number of loci detected in GEA could also mean that strong genomic structure is either masking or hampering the detection of adaptive variants or that different genetic variants are used for adaptive purposes within the defined structure clusters. The observed pattern could also be

partly due to genomic dataset construction – reads from both species were mapped to a reference *P. siculus* genome and variant calling parameterization specified that all loci need to be called in both species. Adaptation in *P. melisellensis* may thus simply be governed by loci that were missed because they were too different for successful mapping on *P. siculus* genome, or are driven by distinct selective pressures that were not explored in this study. More in depth analysis on dataset constructed specifically for *P. melisellensis* should therefore be employed to study the basis of genomic adaptation in these populations. Further GEA studies should also exclude Veli Barjak because of the potential bias introduced by extreme genomic differentiation and signs of genomic introgression from *P. siculus*, which could have negative influence on the estimation of underlying neutral variance across investigated *P. melisellensis* populations (de Villemereuil et al., 2014; Gautier, 2015).

Although genomic differentiation in *P. siculus* also followed the same isolation-by-distance pattern as *P. melisellensis*, multivariate RDA partitioning of variance showed differences in habitat ecology had larger effect on genomic variance in *P. siculus* than geographic distance itself. Significant genotype-environment association was also recorded for multiple tested ecological variables across the three univariate Bayesian GEA analyses used in this study. Not surprisingly, temperature appears to be a major driver of adaptive response across investigated *P. siculus* populations. These result are in accordance with recent studies which similarly relied on Bayenv2 and LFMM methods to detect a number of loci associated with large scale local adaptation to different thermal environments in green anole, *Anolis carolinensis* (Campbell-Staton et al., 2016) and large-headed anole, *Anolis cybotes* (Rodríguez et al., 2017) lizard species. In fact, as shown by Campbell-Staton et al. (2017) who measured cold tolerance performance in wild *A. carolinensis* populations before and after an extreme cold snap throughout southern USA, decrease in minimum temperature during prolonged extreme winter conditions can exert strong selection pressure leading to rapid shift in phenotypic and genomic variation in affected populations. Tightly linked to changes in temperature regimes, precipitation and solar radiation are similarly known to play a key role in lizards physiological performance, with often significant effect on behaviour, population dynamics and local adaptation (Masó et al., 2020; Ortega et al., 2019; Prates et al., 2018). Extreme wind patterns have also been shown to have a potentially strong impact on wild lizard populations. For instance Donihue et al. (2018), who had the opportunity to study phenotypic variation in *Anolis scriptus* lizard species immediately before and after a hurricane event, determined that survivors had significantly longer legs and larger toepads

which improved their clinging ability. Subsequent research further suggested these extreme events might have an enduring evolutionary impact on morphological and phylogenetic patterns in Caribbean anoles in general (Donihue et al., 2020). Though not quite as extreme, the investigated area of Adriatic coast is known for strong north-eastern wind bora, which is characterised by sudden drops in temperature and gusts of wind frequently exceeding 200 km/h, that may exert similar selection pressure on the local fauna. Several other important factors which were not assessed here, such as vegetation cover or predator abundance (Lortie et al., 2020; Vervust et al., 2007) could have an additional effect on adaptation on *P. siculus* populations, along with Pod Mrčaru and Pod Kopište and should be taken into account in future analyses.

5.8 Adaptive nature of Pod Mrčaru and Pod Kopište *P. siculus* divergence

In total, 46 loci were identified as putative candidates for selection in Pod Mrčaru and Pod Kopište *P. siculus* populations across multiple genome scan methods employed to test adaptive divergence between those two populations. The adaptive nature of those candidate loci was further addressed by 1) investigating their contribution to variance in phenotypic traits of interest in two focal populations, and 2) their role in environmental adaptation across all investigated *P. siculus* populations. Notably, LFMM genotype-phenotype association analysis revealed that 17 out of 46 candidate loci showed significant interactions with rapidly diverging phenotypic traits of body, head and limb size in female and male individuals from the two focal populations. The observed trend is indicative of polygenic selection affecting allele frequency distribution across multiple candidate loci underlying adaptive phenotypes (Fuller et al., 2020; Perreault-Payette et al., 2017; Rellstab et al., 2015). These results are therefore further in accordance with the patterns observed from the crossing experiment in the common garden which pointed towards high heritability and large additive genetic component underlying divergent traits connected to head shape in these two populations.

In both Bayenv2 and Baypass genotype-environment association analyses 4 out of 46 of candidate loci for selection in Pod Mrčaru and Pod Kopište were further associated with differences in ecological parameters across all investigated *P. siculus* populations. Markedly, Baypass core model analysis based on XtX statistics likewise revealed that 6 out of 46 candidate loci for selection in two focal populations also show signatures of selection across all analysed *P. siculus* populations. Furthermore, RDA analysis on all *P. siculus* populations conducted using only 46 candidate loci for selection in Pod Mrčaru and Pod Kopište

explained more ecological variation than the analysis on *P. siculus* dataset containing all genotyped loci, suggesting their allelic frequencies are more driven by environmental selection than the genome-wide average (Bernatchez et al., 2019; Capblancq et al., 2018). The results of those three different analytical approaches build up a strong indication that some loci detected as putatively under selection in Pod Mrčaru and Pod Kopište are also adaptive and driven by selection in *P. siculus* in general. On the other hand, no evolutionary parallelism in adaptive response was detected between *P. siculus* and *P. melisellensis* populations, and 46 candidate loci for selection identified in the two focal populations did not appear important for adaptation in *P. melisellensis*.

The observed patterns are consistent with signatures of rapid adaptation from standing genomic variation (Barrett and Schluter, 2008; Dayan et al., 2019; Lai et al., 2019). Obtained results thereby indicate that rapid phenotypic divergence of Pod Mrčaru *P. siculus* population is not driven by phenotypic plasticity alone, but also by genetically based adaptation to different ecological conditions encountered in the novel habitat.

6. CONCLUSIONS

1) *Phenotypic traits related to head shape are moderately to highly heritable in Pod Mrčaru and Pod Kopište P. siculus populations.*

The results presented in this study point towards large additive genetic component underlining variability in phenotypic traits connected to head size and shape in *P. siculus* populations from islets of Pod Mrčaru and Pod Kopište. Though the persistence of phenotypic divergence in individuals raised in common garden was not confirmed, the investigated traits themselves are heritable and possess enough additive variance to evolve under selection pressure encountered by individuals transplanted to the novel environment.

2) *Pod Mrčaru and Pod Kopište P. siculus populations are genomically differentiated.*

Clear genomic divergence was found between Pod Mrčaru and Pod Kopište *P. siculus* populations. Moreover, the levels of genomic diversity in Pod Mrčaru *P. siculus* population were not notably decreased, and no signs of increased inbreeding were detected.

3) *Differentiation between Pod Mrčaru and Pod Kopište P. siculus population is in part driven by rapid genomic adaptation.*

Adaptive nature of loci identified as putatively under selection in Pod Mrčaru and Pod Kopište populations was corroborated by their association with divergent phenotypic traits in these two populations, as well as their importance for polygenic local adaptation in *P. siculus* in general. The results presented in this study thus suggest that rapid genomic adaptation from standing genetic variation is contributing to phenotypic, ecological and genomic divergence of Pod Mrčaru *P. siculus* population.

This study uses integrative scientific approach and combines quantitative genetic and population genomic methods to demonstrate that genomic adaptation may proceed rapidly in face of strong evolutionary pressure. Presented results thus contribute to the fast growing evidence of eco-evolutionary interactions occurring on contemporary scales, which are becoming of high concern for conservation and preservation of wild populations in increasingly fast-changing natural ecosystems. The obtained insights may serve to inform future research focusing on the specifics of molecular processes underling genomic adaptation and population persistence, both in this system and small isolated populations in general.

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8. SUPPLEMENTARY MATERIAL

8.1 Materials and methods

8.1.1 Phylogenetic analysis

Phylogenetic analysis was performed on 20 wild *Podarcis* populations sampled in 2016 (Figure 5) in order to evaluate differences in lizards performance independent from phylogenetic effects (for more details see Taverne et al., 2020). Mitochondrial cytochrome *b* sequences were obtained from GenBank for 14 out of the 20 studied populations. Optimized protocols from Podnar et al. (2004, 2005) were used to obtain mitochondrial cytochrome *b* sequences for 6 populations not represented in GenBank (Bijelac, Kopište and Pod Mrčaru, Sinj, Veli Barjak and Veli Tajan). For both *P. siculus* and *P. melisellensis* populations, 40–50 ng of extracted genomic DNA was amplified in a 25 μL reaction containing 5× Iproof buffer, nfH_20, 1.5 mm $MgCl_2$, 0.2 mm of each dNTP, 0.4 μm of each primer and 0.5 U iProof Polymerase (Bio-Rad). PCR conditions included initial denaturation for 2 min at 98 °C, followed by 35 cycles of 10 s at 98 °C, 20 s at 50 °C and 90 s at 72 °C, and a final extension of 7 min at 72 °C. Samples from Bijelac, Kopište and Pod Mrčaru were further reamplified using mitochondrial specific primer to account for the presence of cytochrome *b* nuclear pseudogene sequences (numts) in the *P. siculus* populations (Podnar et al., 2005). Reamplification was performed using 1 μL of the amplification mix and included the same conditions as the amplification, apart from the primers used, reaction volume which was set to 50 μL and the PCR annealing temperature that was adjusted to 55 °C. All PCRs were performed in a Bio-Rad Gradient Thermal Cycler. Macrogen (Amsterdam, Netherlands) provided PCR product purification and bidirectional sequencing using the primers listed in Table S1.

Chromatograms were loaded in Geneious 4.8.5 (Biomatters, Auckland, New Zealand), corrected manually, aligned and trimmed to the same length together with the sequences from the other previously published populations. The optimal nucleotide substitution model was determined with jModelTest 2.1.10 (Darriba et al., 2012; Guindon and Gascuel, 2003). Likelihood scores were computed using the 'best' base tree topology search method (from both 'Nearest Neighbour Interchange' and 'Subtree Pruning and Regrafting'). MEGA-X 10.0.5 (Kumar et al., 2018) was used to calculate nucleotide composition, nucleotide pair frequencies, and transition and transversion rates (R ratio) according to the chosen nucleotide

substitution model. Cytochrome *b* sequence from the Adriatic lineage of *Lacerta viridis* complex from Krbavica (Croatia) was added as an outgroup before analysis (Marzahn et al., 2016). Phylogenetic trees were generated using maximum parsimony (MP) method implemented in PAUP 4.0a (Swofford and Sullivan, 2003). MP analysis was performed using the chosen nucleotide substitution model and calculated R ratio, with full heuristic search, tree-bisection-reconnection (TBR) branch-swapping and 1000 bootstrap replicates. Starting trees were obtained via stepwise addition, with ten replicates of each random addition sequence.

Table S1 List of primers used for cytochrome *b* sequence analysis. Primer use: A – PCR amplification, R – PCR reamplification, and S – sequencing.

Species	Primer ID	Primer sequence 5′ 3′	Primer use
P. siculus	**L-14253**	TTTGGATCCCTGTTAGGCCTCTGCC	A, R
	H-15425	GGTTTACAAGACCAGTGCTTT	A
	H-15150	ATAATAAAGGGGTGTTCTACTGGTTGGCC	R, S
	H-14776	GGTGGAATGGGATTTTGTCTG	S
P. melisellensis	**L-14132**	ATTCAACTATTAAAACCTCTAATG	A
	H-15425	GGTTTACAAGACCAGTGCTTT	A
	H-15150	ATAATAAAGGGGTGTTCTACTGGTTGGCC	S
	H-14776	GGTGGAATGGGATTTTGTCTG	S

8.1.2 Reference genome assembly

Podarcis siculus reference genome, assembled from an individual from Pod Mrčaru, was provided by Rasmus Nielsen research group from Berkeley University in the USA.

Table S2 *Podarcis siculus* genome assembly characteristics.

Genome feature	Value
Number of scaffolds >= 10 kb	1.17 K
N50 edge size	13.46 Kb
N50 contig size	75.56 Kb
N50 phase block size	1.11 Mb
N50 scaffold size	37.45 Mb
% of base assembly missing from scaffolds >= 10 kb	3.57 %
Assembly size (only scaffolds >= 10 kb)	1.33 Gb

8.1.3 Quality control, alignment and variant detection

Table S3 Number of samples (n) genotyped per site and species across all investigated *Podarcis* populations.

ID	site	species	n
BJ	Bijelac	*P. siculus*	**19**
DU	Veli Dupinić	*P. siculus*	**25**
KL	Kluda	*P. siculus*	**22**
KP	Kopište	*P. siculus*	**30**
OB	Obrovanj	*P. siculus*	**25**
OS	Oštrica	*P. siculus*	**25**
PG	Mala Palagruža	*P. siculus*	**20**
PJ	Pijavica	*P. siculus*	**20**
PK	Pod Kopište	*P. siculus*	**43**
PM	Pod Mrčaru	*P. siculus*	**46**
RK	Rakita	*P. siculus*	**25**
SC	Sušac	*P. siculus*	**19**
ST	Split	*P. siculus*	**18**
VC	Visovac	*P. siculus*	**25**
BD	Budikovac	*P. melisellensis*	**19**
BR	Brusnik	*P. melisellensis*	**28**
GL	Glavat	*P. melisellensis*	**10**
GN	Grebeni	*P. melisellensis*	**20**
JK	Jabuka	*P. melisellensis*	**20**
KM	Kamik	*P. melisellensis*	**15**
MB	Mali Barjak	*P. melisellensis*	**20**
PZ	Paržanj	*P. melisellensis*	**20**
RV	Ravnik	*P. melisellensis*	**20**
SN	Sinj	*P. melisellensis*	**17**
VB	Veli Barjak	*P. melisellensis*	**29**
VT	Veli Tajan	*P. melisellensis*	**20**

8.1.4 Genotype-environment associations

Table S4 Raw values of environmental variables obtained for 14 sampling locations of *P. siculus* populations. Variable abbreviations are defined in Table 2, and population abbreviations in Figure 5.

	BJ	DU	KL	KP	OB	OS	PG	PJ	PK	PM	RK	SC	ST	VC
Longitude	16.68	15.71	16.17	16.72	15.50	15.71	16.27	16.19	16.72	16.77	15.50	16.50	16.44	15.54
Latitude	42.76	43.71	43.48	42.75	43.84	43.70	42.39	43.48	42.76	42.78	43.84	42.76	43.51	43.83
Area	5530	16075	78407	738726	40002	20648	26510	11037	35835	13514	4001	4025460	10000000	17376
Altitude	16	12	50	93	10	10	51	8	30	16	5	239	89	25
DistanceToLand	45484	5937	845	41612	5763	7143	106705	2619	41270	35853	5840	64350	0	4062
DistanceToLargeIsland	13023	5937	845	7230	5763	7143	72250	2619	7930	3021	5840	29141	0	4062
DephToCoastMax	91.20	59.00	0.20	85.20	4.83	61.40	226.00	6.59	86.20	86.20	4.34	113.20	0.00	14.02
TemperatureMean	16.75	15.71	16.01	16.75	15.80	15.72	16.68	16.29	16.75	16.20	15.69	16.45	16.36	15.57
TemperatureMin	6.1	4.3	5	6.1	5.4	4.4	6.7	4.7	6.1	5.3	4.4	5.1	4.2	4.5
TemperatureMax	26.7	26.1	27.4	26.7	25.8	26.1	26.7	28.5	26.7	26	26.3	27.1	28.7	25.7
TemperatureRange	20.6	21.8	22.4	20.6	20.4	21.7	20	23.8	20.6	20.7	21.9	22	24.5	21.2
PrecipitationTotal	617	692	703	617	746	689	438	713	617	632	757	570	777	744
PercipitationMean	51.42	57.67	58.58	51.42	62.17	57.42	36.5	59.42	51.42	52.67	63.08	47.5	64.75	62
PrecipitationMin	16	23	25	16	26	23	15	26	16	17	26	17	32	26
PrecipitationMax	79	85	91	79	89	85	57	91	79	80	91	75	100	89
PrecipitationRange	63	62	66	63	63	62	42	65	63	63	65	58	68	63
SolarRadiationTotal	175441	171079	171855	175441	169219	171099	175724	171049	175441	175165	169151	175215	170133	169412
SolarRadiationMean	14620	14257	14321	14620	14102	14258	14644	14254	14620	14597	14096	14601	14178	14118
SolarRadiationMin	4949	4585	4679	4949	4481	4630	4993	4641	4949	4913	4461	4881	4648	4488
SolarRadiationMax	25441	25263	25157	25441	24937	25138	25043	24974	25441	25372	24981	25253	24816	25007
SolarRadiationRange	20492	20678	20478	20492	20456	20508	20050	20333	20492	20459	20520	20372	20168	20519
WindSpeedMean	3.7	3.0	3.2	3.7	2.9	3.0	4.2	3.2	3.7	3.7	2.9	3.8	3.1	2.9
WindSpeedMin	2.9	2.5	2.6	2.9	2.4	2.5	3.3	2.6	2.9	2.9	2.4	3	2.5	2.4
WindSpeedMax	4.5	3.5	3.8	4.5	3.4	3.5	5	3.7	4.5	4.5	3.4	4.6	3.6	3.4
WindSpeedRange	1.6	1	1.2	1.6	1	1	1.7	1.1	1.6	1.6	1	1.6	1.1	1

Table S5 Raw values of environmental variables obtained for 12 sampling locations of *P. melisellensis* populations. Variable abbreviations are defined in Table 2, and population abbreviations in Figure 5.

	BD	BR	GL	GN	JK	KM	MB	PZ	RV	SN	VB	VT
Longitude	16.24	15.80	17.15	16.27	15.46	15.71	16.04	16.26	16.23	16.64	16.04	16.99
Latitude	43.03	43.01	42.77	43.05	43.09	43.02	43.05	43.04	43.02	43.70	43.05	42.82
Area	316748	49455	18430	51690	22585	7738	6232	13403	226605	10000000	18116	20127
Altitude	35	30	20	32	96	40	4	4	38	326	6	15
DistanceToLand	68938	76617	31186	66228	83668	78035	64473	68217	70058	0	64143	20265
DistanceToLargeIsland	957	27750	22201	1208	65305	37235	848	901	545	0	260	7671
DephToCoastMax	120.60	132.20	79.00	123.20	150.40	134.40	125.20	120.60	121.80	0.00	124.80	70.60
TemperatureMean	16.59	15.71	16.97	16.50	15.93	15.93	16.21	16.53	16.71	12.91	16.10	16.79
TemperatureMin	5	4.3	6.2	5.2	4.5	4.5	4.5	5	5	-2.2	4.5	5.1
TemperatureMax	28.3	26.1	28	27.9	26.4	26.4	27.1	28.1	28.5	27.7	26.9	28.3
TemperatureRange	23.3	21.8	21.8	22.7	21.9	21.9	22.6	23.1	23.5	29.9	22.4	23.2
PrecipitationTotal	621	523	745	629	521	526	572	626	612	906	575	701
PercipitationMean	51.75	43.58333	62.08333	52.41667	43.41667	43.83333	47.66667	52.16667	51	75.5	47.91667	58.41667
PrecipitationMin	20	18	21	20	18	18	19	20	20	43	19	19
PrecipitationMax	82	67	95	84	67	68	75	83	81	114	76	89
PrecipitationRange	62	49	74	64	49	50	56	63	61	71	57	70
SolarRadiationTotal	173073	174586	173933	173392	174470	174423	174549	173265	173778	166359	174455	174657
SolarRadiationMean	14422.75	14548.83	14494.42	14449.33	14539.17	14535.25	14545.75	14438.75	14481.5	13863.25	14537.92	14554.75
SolarRadiationMin	4795	4840	4847	4806	4798	4816	4800	4810	4815	4566	4805	4915
SolarRadiationMax	25213	25252	25333	25218	25333	25333	25358	25214	25284	24405	25283	25373
SolarRadiationRange	20418	20412	20486	20412	20535	20517	20558	20404	20469	19839	20478	20458
WindSpeedMean	3.54	3.62	3.55	3.53	3.57	3.58	3.49	3.53	3.53	2.18	3.53	3.59
WindSpeedMin	2.9	2.9	2.8	2.9	2.9	2.9	2.9	2.9	2.9	1.8	2.9	2.8
WindSpeedMax	4.2	4.3	4.2	4.2	4.2	4.2	4.1	4.2	4.2	2.6	4.2	4.3
WindSpeedRange	1.3	1.4	1.4	1.3	1.3	1.3	1.2	1.3	1.3	0.8	1.3	1.5

8.2 Results

8.2.1 Repeatability of the phenotypisation procedure for individuals from the common garden experiment

Table S6 Repeatability (R) of the phenotypisation procedure (photographing and extracting phenotypic measure using geometric morphometry based on landmark data) estimated from 9 technical replicates (3 pictures * 3 replicates per picture) obtained for 21 F1 individuals re-phenotyped in October 2020. One-way ANOVA was performed on all 9 replicates for each trait using individuals as categorical variable. Repeatability (R) was estimated as $R = S^2_A/(S^2_W+S^2_A)$, where the within-individual variation S^2_W is given by $MS_{Residual}$ and among-individual variation S^2_A is calculated as $S^2_A = (MS_{Individual} - MS_{Residual})/n$, where n is the number of replicates per individual (Arnqvist and Mårtensson, 1998). Phenotypic trait abbreviations are defined in the text (Df = degrees of freedom, SS = sum of squares, MS = mean squares).

	Df	SS	MS	F value	P value	R
			HHght			
Individual	20	1.2279	0.06140	89.97	<2e-16 ***	0.908
Residuals	168	0.1146	0.00068			
			HLgth			
Individual	20	4.857	0.24284	89.29	<2e-16 ***	0.907
Residuals	168	0.457	0.00272			
			HWdth			
Individual	20	2.6453	0.13226	70.35	<2e-16 ***	0.885
Residuals	168	0.3159	0.00188			
			LwJaL			
Individual	20	4.719	0.23596	89.83	<2e-16 ***	0.908
Residuals	168	0.441	0.00263			
			LwJaO			
Individual	20	4.685	0.23423	96.71	<2e-16 ***	0.914
Residuals	168	0.407	0.00242			
			SnLgh			
Individual	20	2.1952	0.10976	51.82	<2e-16 ***	0.850
Residuals	168	0.3558	0.00212			
			LtHip			
Individual	20	39.04	1.9521	30.28	<2e-16 ***	0.765
Residuals	168	10.83	0.0645			
			TailL			
Individual	20	875	43.75	341.5	<2e-16 ***	0.974
Residuals	168	21.5	0.13			

8.2.2 Heritability estimation

Table S7 Maximum log likelihoods (ASSOC and WOMBAT) and deviance information criterion (DIC; MCMCglmm) obtained for simple models considering only pedigree component, and extended models accounting for sex, experimental year, and parental source population parameters. NA marks ASSOC analyses for which convergence was not achieved. Phenotypic trait abbreviations are defined in the text.

	simple model			*sex + year +population extended model*			*year + population extended model*		
	ASSOC	**WOMBAT**	**MCMCglmm**	**ASSOC**	**WOMBAT**	**MCMCglmm**	**ASSOC**	**WOMBAT**	**MCMCglmm**
HHght	-1077.71	385.46	-555.74	-1073.16	372.520	-589.5718	-1074.83	363.64	-584.30
HLgth	-1078.21	221.57	-206.63	-1069.55	231.653	-281.5172	-1077.65	205.95	-307.10
HWdth	-1078.63	335.32	-461.53	-1066.5	370.955	-506.6489	-1077.70	311.58	-477.41
LwJaL	-1077.91	224.92	-201.01	-1069.30	232.543	-266.7698	-1077.80	208.50	-258.38
LwJaO	-1077.25	223.52	-198.35	-1068.62	227.749	-252.8507	-1077.24	207.59	-249.89
SnLgh	-1074.14	335.58	-428.60	NA	325.312	-475.2119	-1074.30	314.99	-483.23
BiteF	NA	-148914.04	593.04	NA	-16757.923	576.6127	NA	-40275.96	582.78
LtHip	NA	167.50	-20.11	NA	240.310	-263.3798	NA	236.22	-248.74
TailL	-1077.39	-40259741.39	1378.35	-1078.55	-32024118.082	1381.567	-1078.33	-32577992.99	1382.91

8.2.3 Genomic differentiation

Table S8 Population pairwise F_{ST} values obtained for all 26 wild *Podarcis* populations. All calculations were significant (all p-values = 0 after 99999 bootstrap iterations). Population abbreviations are defined in Figure 5.

	PG	SC	BJ	KP	PK	PM	PJ	KL	OS	DU	VC	OB	RK	ST	SN	GL	VT	GN	PZ	BD	RV	VB	MB	BR	KM	JK	
PG		0.18	0.37	0.21	0.27	0.29	0.30	0.33	0.67	0.69	0.64	0.57	0.60	0.58	0.94	0.95	0.96	0.94	0.95	0.94	0.95	0.92	0.97	0.96	0.96	0.96	**PG**
SC	0.18		0.30	0.07	0.12	0.16	0.27	0.32	0.71	0.73	0.67	0.60	0.63	0.63	0.95	0.96	0.97	0.95	0.96	0.95	0.96	0.93	0.98	0.97	0.97	0.97	**SC**
BJ	0.37	0.30		0.26	0.30	0.34	0.43	0.46	0.75	0.78	0.72	0.65	0.68	0.69	0.96	0.98	0.98	0.96	0.97	0.96	0.97	0.94	0.99	0.98	0.99	0.98	**BJ**
KP	0.21	0.07	0.26	-	0.08	0.12	0.30	0.35	0.73	0.75	0.70	0.64	0.66	0.66	0.95	0.96	0.97	0.95	0.96	0.96	0.96	0.93	0.97	0.97	0.97	0.97	**KP**
PK	0.27	0.12	0.30	0.08		0.04	0.36	0.40	0.76	0.77	0.73	0.68	0.70	0.70	0.96	0.96	0.97	0.96	0.96	0.96	0.96	0.94	0.97	0.97	0.97	0.97	**PK**
PM	0.29	0.16	0.34	0.12	0.04		0.38	0.43	0.77	0.78	0.74	0.69	0.71	0.71	0.96	0.97	0.97	0.96	0.97	0.96	0.96	0.94	0.98	0.97	0.97	0.97	**PM**
PJ	0.30	0.27	0.43	0.30	0.36	0.38		0.15	0.66	0.68	0.62	0.55	0.58	0.56	0.94	0.95	0.96	0.94	0.95	0.95	0.95	0.92	0.97	0.96	0.96	0.96	**PJ**
KL	0.33	0.32	0.46	0.35	0.40	0.43	0.15		0.64	0.66	0.60	0.53	0.56	0.54	0.93	0.94	0.96	0.94	0.95	0.94	0.94	0.92	0.96	0.96	0.96	0.96	**KL**
OS	0.67	0.71	0.75	0.73	0.76	0.77	0.66	0.64		0.26	0.40	0.29	0.33	0.34	0.92	0.93	0.94	0.92	0.93	0.93	0.93	0.91	0.95	0.94	0.94	0.94	**OS**
DU	0.69	0.73	0.78	0.75	0.77	0.78	0.68	0.66	0.26		0.44	0.34	0.37	0.38	0.93	0.93	0.95	0.93	0.94	0.93	0.94	0.91	0.96	0.95	0.95	0.95	**DU**
VC	0.64	0.67	0.72	0.70	0.73	0.74	0.62	0.60	0.40	0.44		0.24	0.29	0.28	0.91	0.92	0.94	0.92	0.93	0.92	0.93	0.90	0.95	0.94	0.94	0.94	**VC**
OB	0.57	0.60	0.65	0.64	0.68	0.69	0.55	0.53	0.29	0.34	0.24		0.16	0.18	0.90	0.90	0.92	0.91	0.91	0.91	0.91	0.89	0.93	0.93	0.92	0.93	**OB**
RK	0.60	0.63	0.68	0.66	0.70	0.71	0.58	0.56	0.33	0.37	0.29	0.16		0.21	0.91	0.91	0.93	0.91	0.92	0.91	0.92	0.90	0.94	0.93	0.93	0.93	**RK**
ST	0.58	0.63	0.69	0.66	0.70	0.71	0.56	0.54	0.34	0.38	0.28	0.18	0.21		0.90	0.91	0.93	0.91	0.92	0.91	0.92	0.89	0.94	0.94	0.93	0.94	**ST**
SN	0.94	0.95	0.96	0.95	0.96	0.96	0.94	0.93	0.92	0.93	0.91	0.90	0.91	0.90		0.41	0.52	0.61	0.68	0.63	0.66	0.54	0.81	0.74	0.77	0.76	**SN**
GL	0.95	0.96	0.98	0.96	0.96	0.97	0.95	0.94	0.93	0.93	0.92	0.90	0.91	0.91	0.41		0.69	0.67	0.75	0.70	0.73	0.58	0.91	0.81	0.88	0.85	**GL**
VT	0.96	0.97	0.98	0.97	0.97	0.97	0.96	0.96	0.94	0.95	0.94	0.92	0.93	0.93	0.52	0.69		0.75	0.81	0.77	0.79	0.65	0.93	0.85	0.91	0.88	**VT**
GN	0.94	0.95	0.96	0.95	0.96	0.96	0.94	0.94	0.92	0.93	0.92	0.91	0.91	0.91	0.61	0.67	0.75		0.22	0.18	0.23	0.10	0.54	0.61	0.64	0.63	**GN**
PZ	0.95	0.96	0.97	0.96	0.96	0.97	0.95	0.95	0.93	0.94	0.93	0.91	0.92	0.92	0.68	0.75	0.81	0.22		0.31	0.34	0.20	0.67	0.69	0.74	0.72	**PZ**
BD	0.94	0.95	0.96	0.96	0.96	0.96	0.95	0.94	0.93	0.93	0.92	0.91	0.91	0.91	0.63	0.70	0.77	0.18	0.31		0.28	0.13	0.60	0.64	0.68	0.66	**BD**
RV	0.95	0.96	0.97	0.96	0.96	0.96	0.95	0.94	0.93	0.94	0.93	0.91	0.92	0.92	0.66	0.73	0.79	0.23	0.34	0.28		0.17	0.63	0.67	0.72	0.70	**RV**
VB	0.92	0.93	0.94	0.93	0.94	0.94	0.92	0.92	0.91	0.91	0.90	0.89	0.90	0.89	0.54	0.58	0.65	0.10	0.20	0.13	0.17		0.41	0.52	0.53	0.52	**VB**
MB	0.97	0.98	0.99	0.97	0.97	0.98	0.97	0.96	0.95	0.96	0.95	0.93	0.94	0.94	0.81	0.91	0.93	0.54	0.67	0.60	0.63	0.41		0.83	0.92	0.88	**MB**
BR	0.96	0.97	0.98	0.97	0.97	0.97	0.96	0.96	0.94	0.95	0.94	0.93	0.93	0.94	0.74	0.81	0.85	0.61	0.69	0.64	0.67	0.52	0.83		0.64	0.59	**BR**
KM	0.96	0.97	0.99	0.97	0.97	0.97	0.96	0.96	0.94	0.95	0.94	0.92	0.93	0.93	0.77	0.88	0.91	0.64	0.74	0.68	0.72	0.53	0.92	0.64		0.66	**KM**
JK	0.96	0.97	0.98	0.97	0.97	0.97	0.96	0.96	0.94	0.95	0.94	0.93	0.93	0.94	0.76	0.85	0.88	0.63	0.72	0.66	0.70	0.52	0.88	0.59	0.66		**JK**
	PG	**SC**	**BJ**	**KP**	**PK**	**PM**	**PJ**	**KL**	**OS**	**DU**	**VC**	**OB**	**RK**	**ST**	**SN**	**GL**	**VT**	**GN**	**PZ**	**BD**	**RV**	**VB**	**MB**	**BR**	**KM**	**JK**	

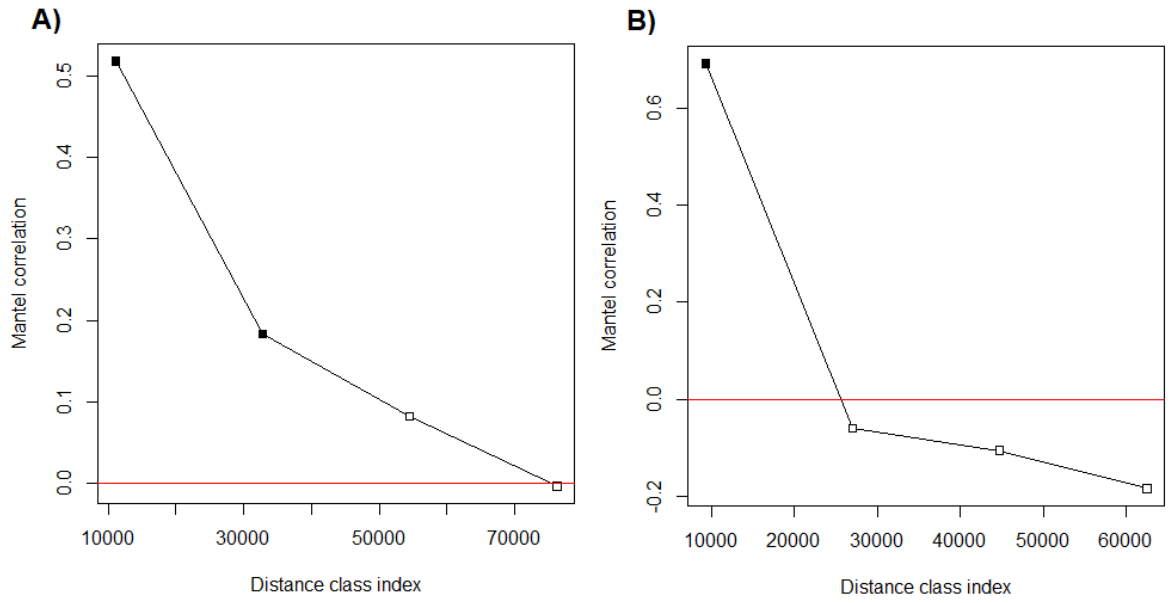

Figure S1 Mantel correlogram for **A)** *P. siculus* (r = 0.72; p = 0.001) and **B)** *P. melisellensis* populations (r = 0.61; p= 0.001).

Table S9 AMOVA results for variance partitioning between *P. siculus* and *P. melisellensis* species and among genomic clusters identified in *fastStructure* analysis. All fixation indices were significant with p < 0.00001.

Source of variation	df	Sum of squares	Variance components	% variation	Fixation indices
Between species	1	3671349.88	6184.97	84.16	F_{CT} = 0.84
Among clusters within species	9	581283.81	685.09	9.32	F_{SC} = 0.59
Among individuals within clusters	589	376528.11	159.87	2.18	F_{IS} = 0.33
Within individuals	600	191717	319.53	4.35	F_{IT} = 0.96

Table S10 AMOVA results for variance partitioning among *P. siculus* populations and genomic clusters identified in *fastStructure* analysis. All fixation indices were significant with p < 0.00001.

Source of variation	df	Sum of squares	Variance components	% variation	Fixation indices
Among clusters	5	375937.44	692.53	56.02	F_{CT} = 0.56
Among populations within clusters	8	42404.97	89.05	7.2	F_{SC} = 0.16
Among individuals within populations	348	185654.92	78.77	6.37	F_{IS} = 0.17
Within individuals	362	136096	375.96	30.41	F_{IT} = 0.7

Table S11 AMOVA results for variance partitioning among *P. melisellensis* populations and genomic clusters identified in *fastStructure* analysis. All fixation indices were significant with $p < 0.00001$.

Source of variation	df	Sum of squares	Variance components	% variation	Fixation indices
Among clusters	4	205346.37	526.76	50.8	$F_{CT} = 0.51$
Among populations within clusters	7	54430.85	185.33	17.87	$F_{SC} = 0.36$
Among individuals within populations	226	94037.37	91.20	8.79	$F_{IS} = 0.28$
Within individuals	238	55621	233.70	22.54	$F_{IT} = 0.77$

8.2.4 Identification of candidate loci for selection

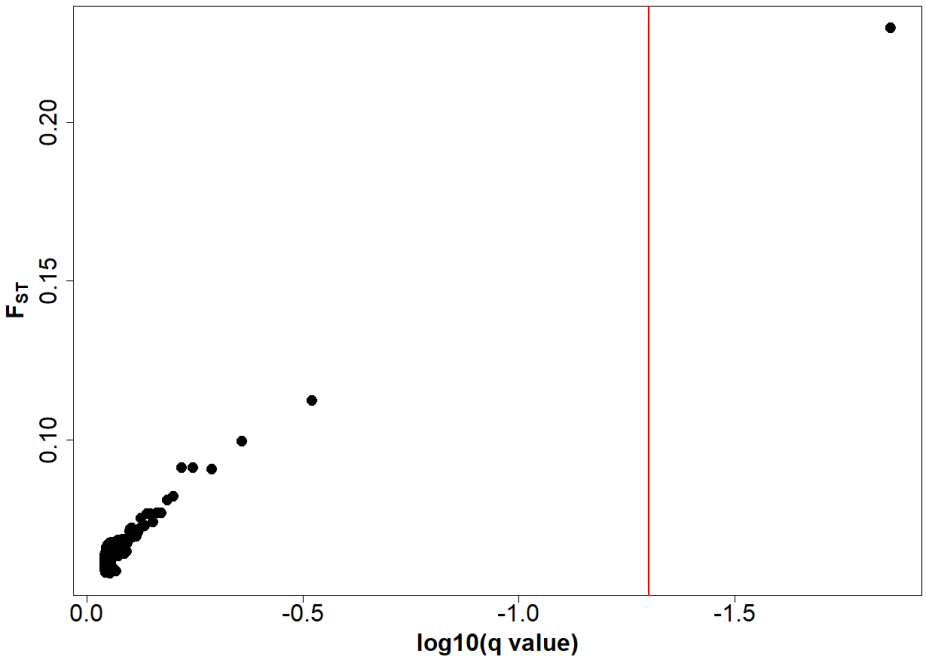

Figure S2 Results of BayeScan analysis for 2421 SNPs genotyped in Pod Mrčaru and Pod Kopište *P. siculus* populations. The marker-specific F_{ST} is plotted against q-value. The vertical line shows the FDR q-value cut-off 0.05 used to identify outlier markers.

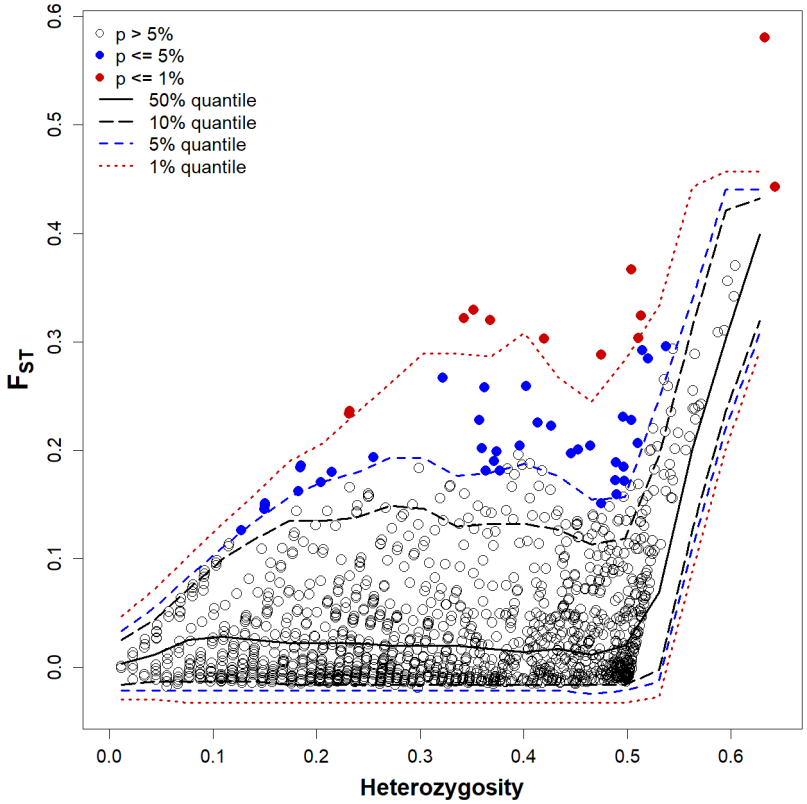

Figure S3 Results of Arlequin analysis of loci under selection for 2421 SNPs genotyped in Pod Mrčaru and Pod Kopište *P. siculus* populations. Blue dashed line shows p-value cut-off 0.05 used to identify outlier markers.

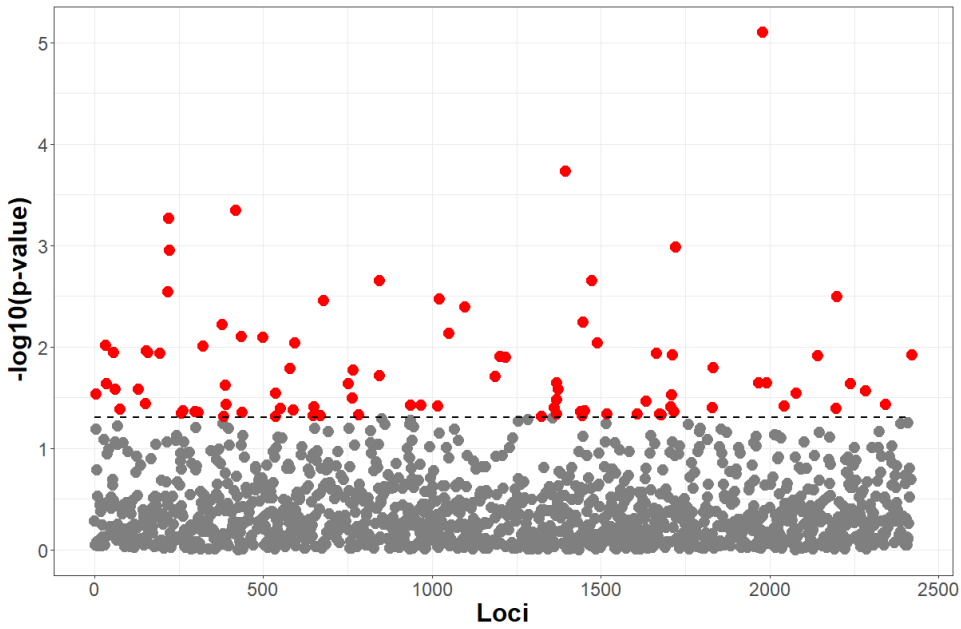

Figure S4 Manhattan plot of *PCAdapt* analysis results for 2421 SNPs genotyped in Pod Mrčaru and Pod Kopište *P. siculus* populations. Black dashed line shows FDR-unadjusted p-value cut-off 0.05 used to identify outlier markers. Outlier loci are marked in red.

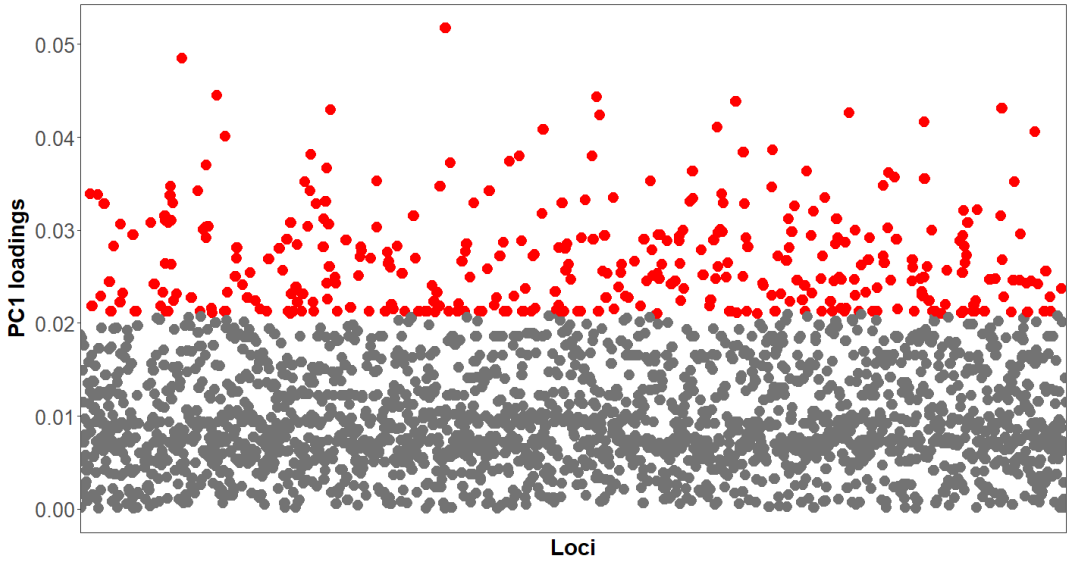

Figure S5 Manhattan plot of PC1 loadings obtained from PCA *adegenet* analysis of 2421 SNPs genotyped in Pod Mrčaru and Pod Kopište *P. siculus* populations. Loci in top 15 % of PC1 loading values were treated as outliers (marked in red).

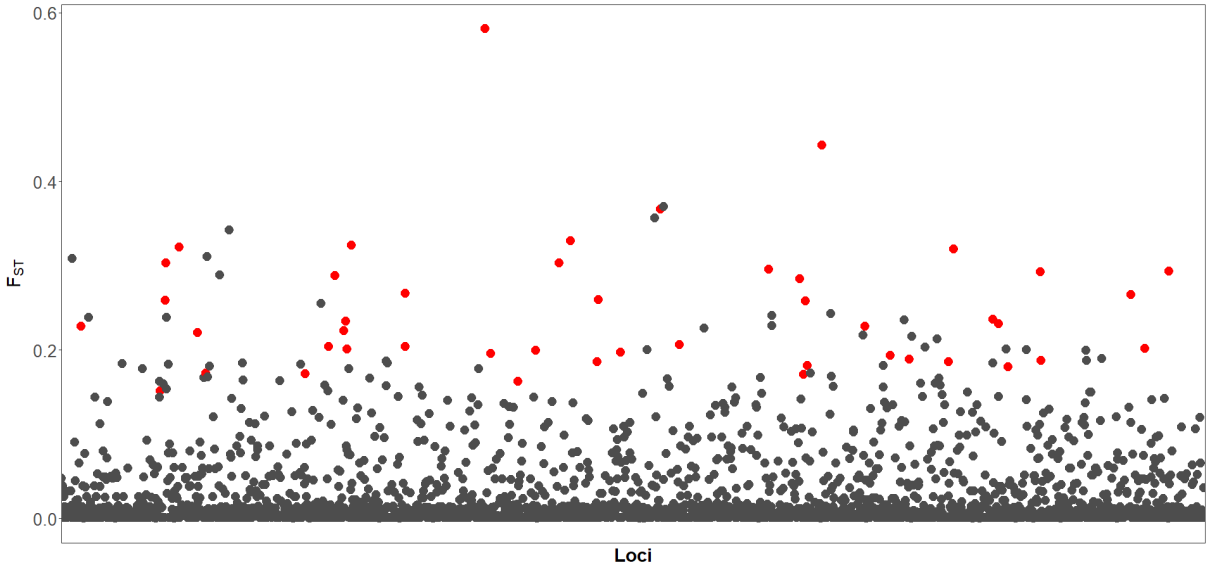

Figure S6 Manhattan plot of loci specific F_{ST} values for 2421 SNPs genotyped in Pod Mrčaru and Pod Kopište *P. siculus* populations. 46 loci identified as putative candidates for selection across Bayescan, Arlequin and/or *PCAdapt* genome scan analyses are in marked in red.

8.2.5 Phenotypic differentiation and genotype-phenotype associations

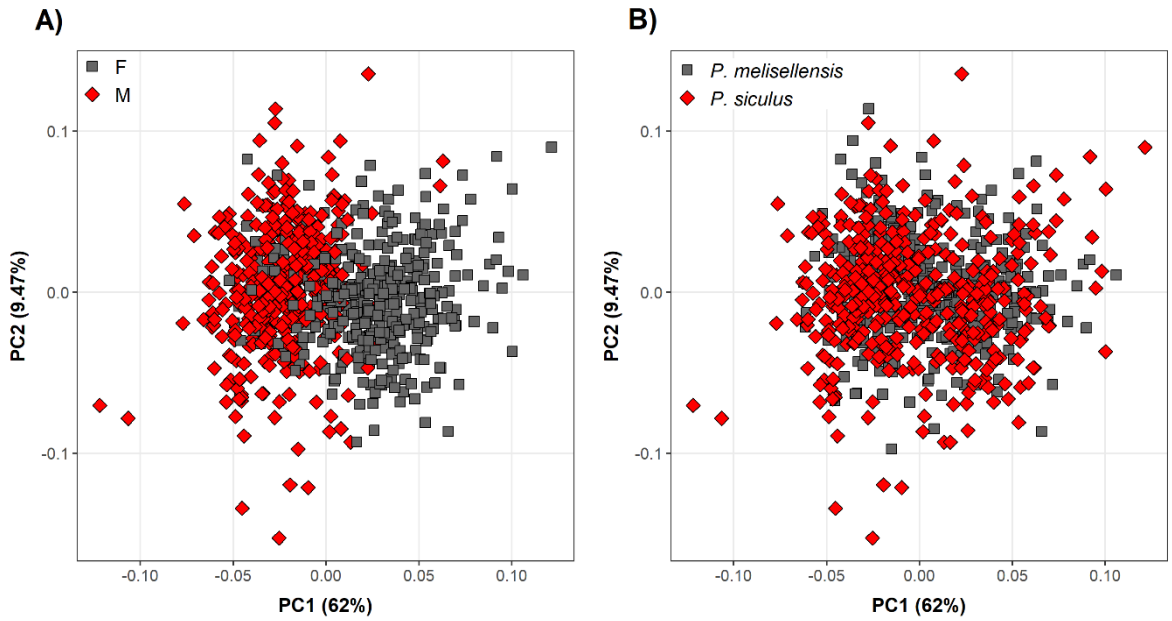

Figure S7 PCA on phenotypic traits obtained from all sampled *Podarcis* individuals, analysed by **A)** sex (F = female, M = male individuals), and **B)** species.

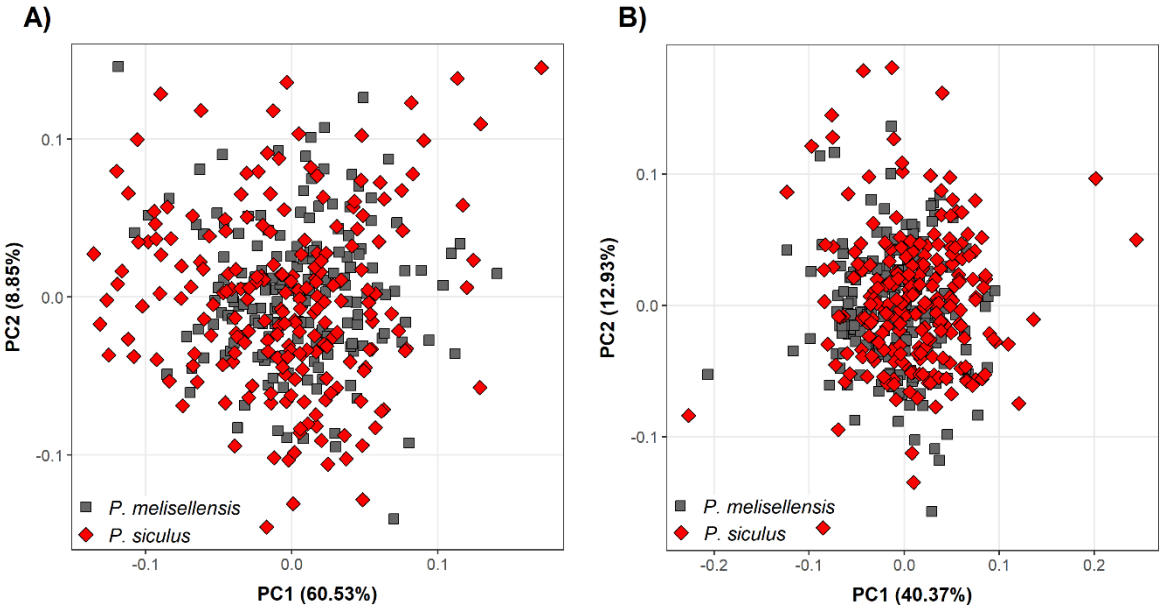

Figure S8 PCA on phenotypic traits obtained from all sampled **A)** female and **B)** male *Podarcis* individuals analysed per species.

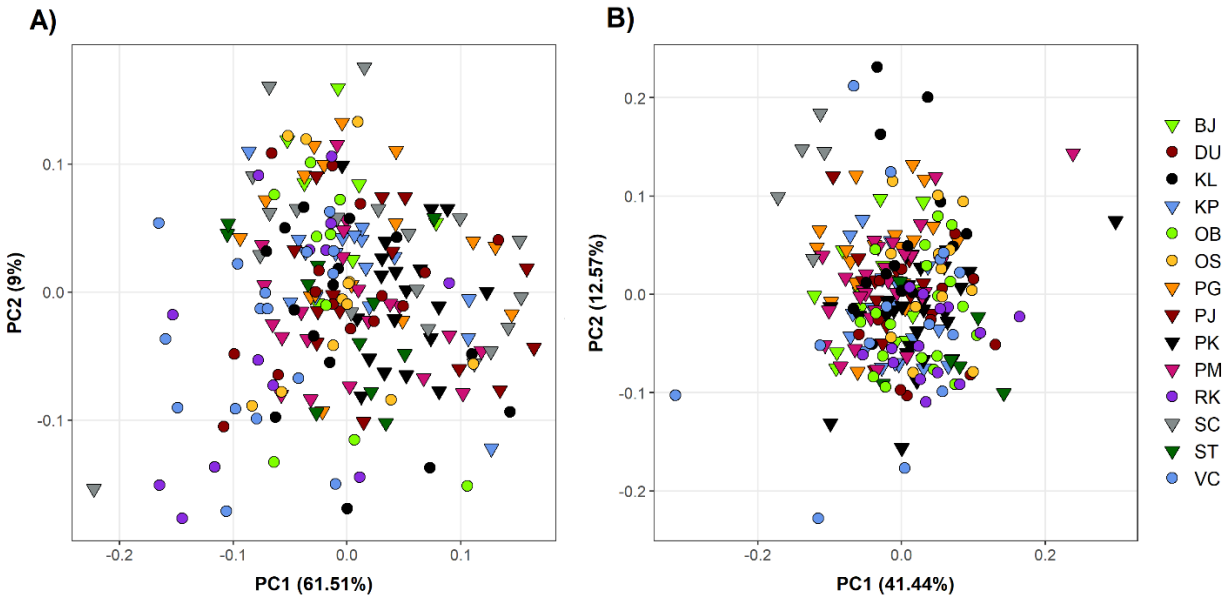

Figure S9 PCA on phenotypic traits obtained from **A)** female and **B)** male *P. siculus* individuals analysed per population.

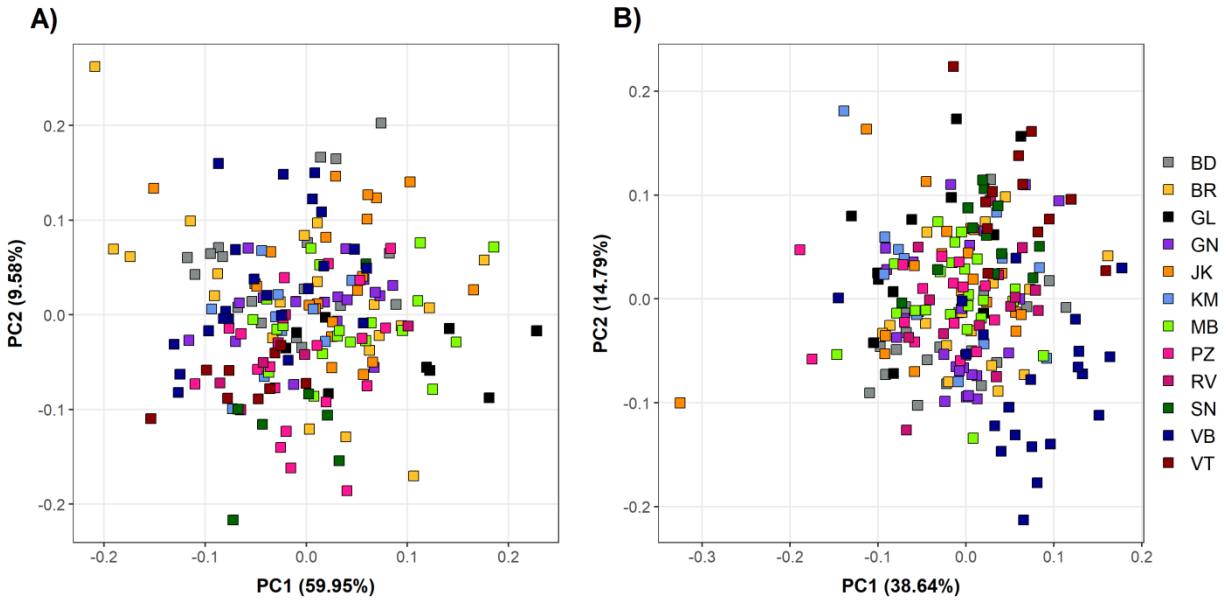

Figure S10 PCA of phenotypic traits obtained from **A)** female and **B)** male *P. melisellensis* individuals analysed per population.

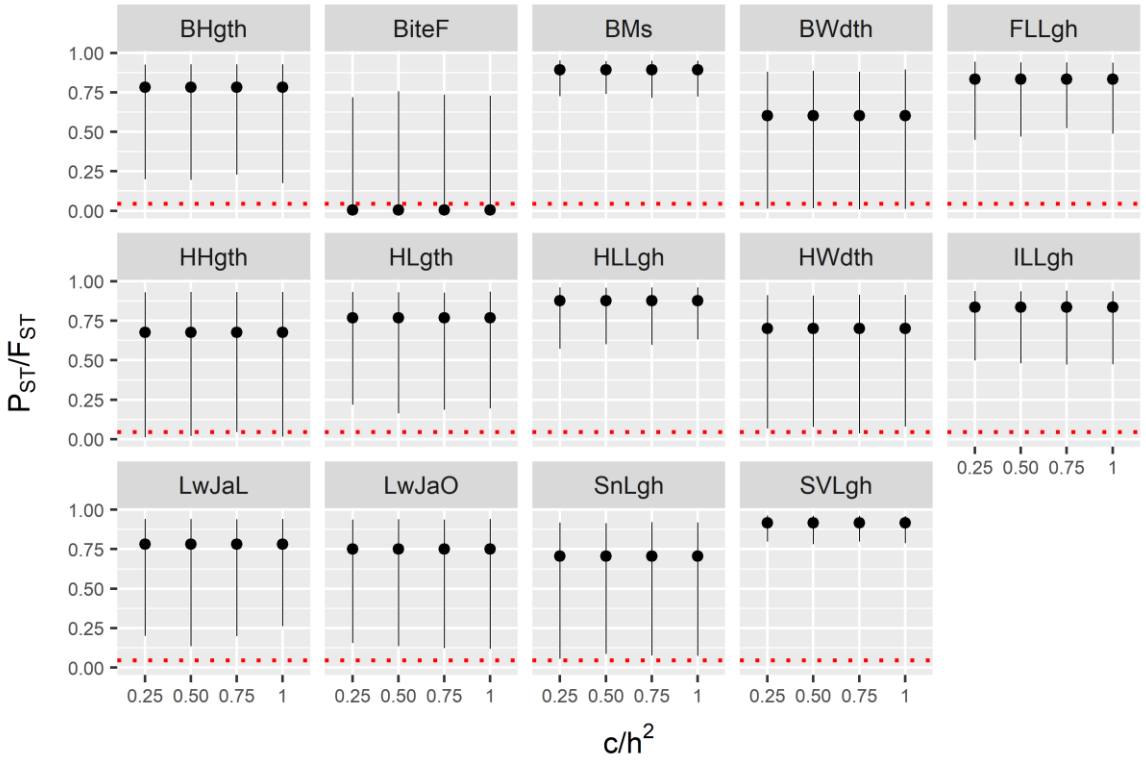

Figure S11 P_{ST} values (black dots) and corresponding confidence intervals (whiskers) compared to population pairwise F_{ST} value (red dashed line) estimated across a range of tested c/h^2 parameter values for female *P. siculus* individuals from Pod Kopište and Pod Mrčaru populations. Phenotypic trait abbreviations are defined in Table 1.

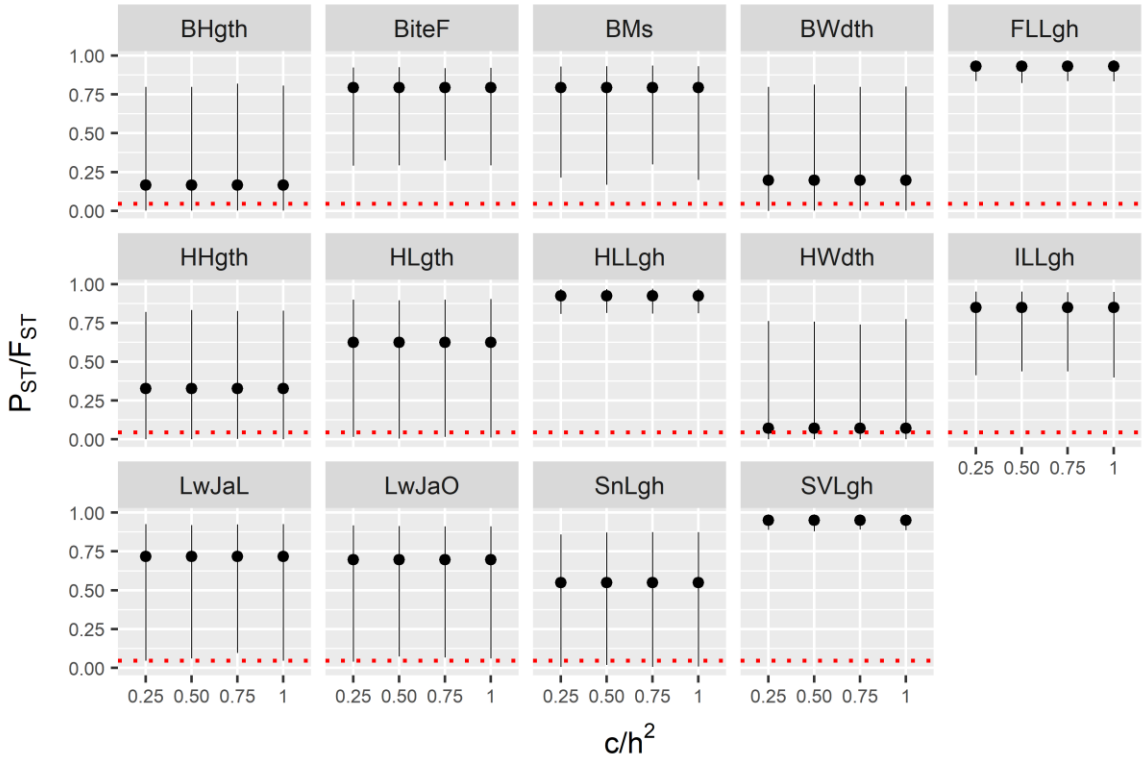

Figure S12 P_{ST} values (black dots) and corresponding confidence intervals (whiskers) compared to population pairwise F_{ST} value (red dashed line) estimated across a range of tested c/h^2 parameter values for male *P. siculus* individuals from Pod Kopište and Pod Mrčaru populations. Phenotypic trait abbreviations are defined in Table 1.

Table S12 Genomic inflation factors (GIFs) obtained during model fitting and modified GIFs used to adjust p-values in LFMM analyses of female and male *P. siculus* individuals from Pod Mrčaru and Pod Kopište islands.

Phenotypic trait	GIF ♀	modified GIF ♀	GIF ♂	modified GIF ♂
SVLgh	1.44	1.00	1.76	1.00
HLgth	1.17	0.60	0.90	0.60
HWdth	1.28	0.65	0.98	0.60
HHgth	0.95	0.70	1.15	0.70
LwJaL	1.03	0.70	1.25	0.60
LwJaO	0.97	0.65	0.90	0.60
SnLgh	0.85	0.70	0.88	0.65
ILLgh	1.20	0.70	1.15	0.75
HLLgh	1.21	0.80	1.47	0.85
FLLgh	1.20	0.85	1.29	0.90
BHgth	1.25	0.75	0.87	0.65
BWdth	1.18	0.85	0.93	0.70
BMs	1.49	0.90	1.12	0.65
BiteF	1.17	0.60	1.27	0.75

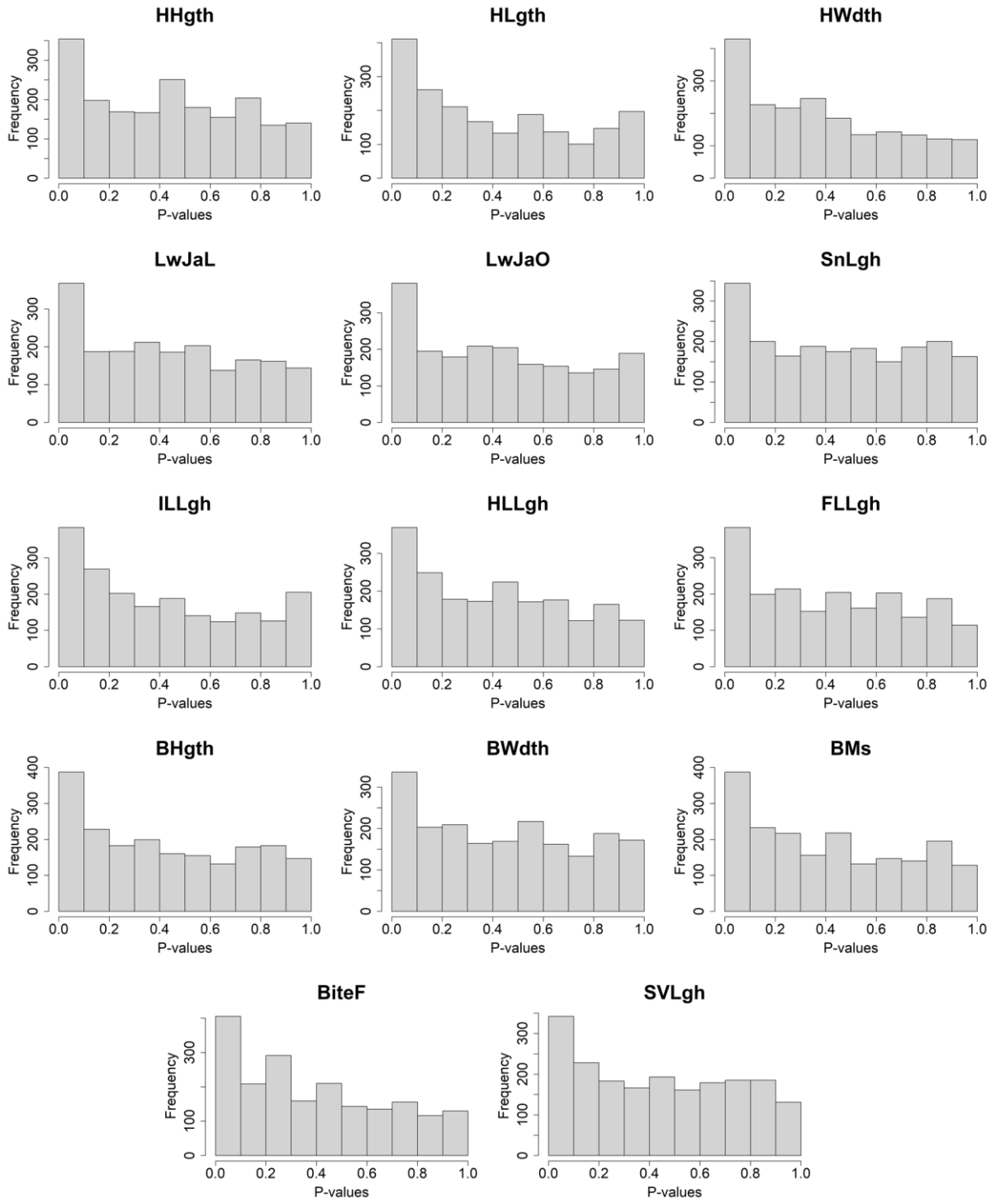

Figure S13 Histograms of adjusted p-values for all loci found in LFMM analysis of female *P. siculus* individuals from Pod Mrčaru and Pod Kopište islands. Phenotypic trait abbreviations are defined in Table 1.

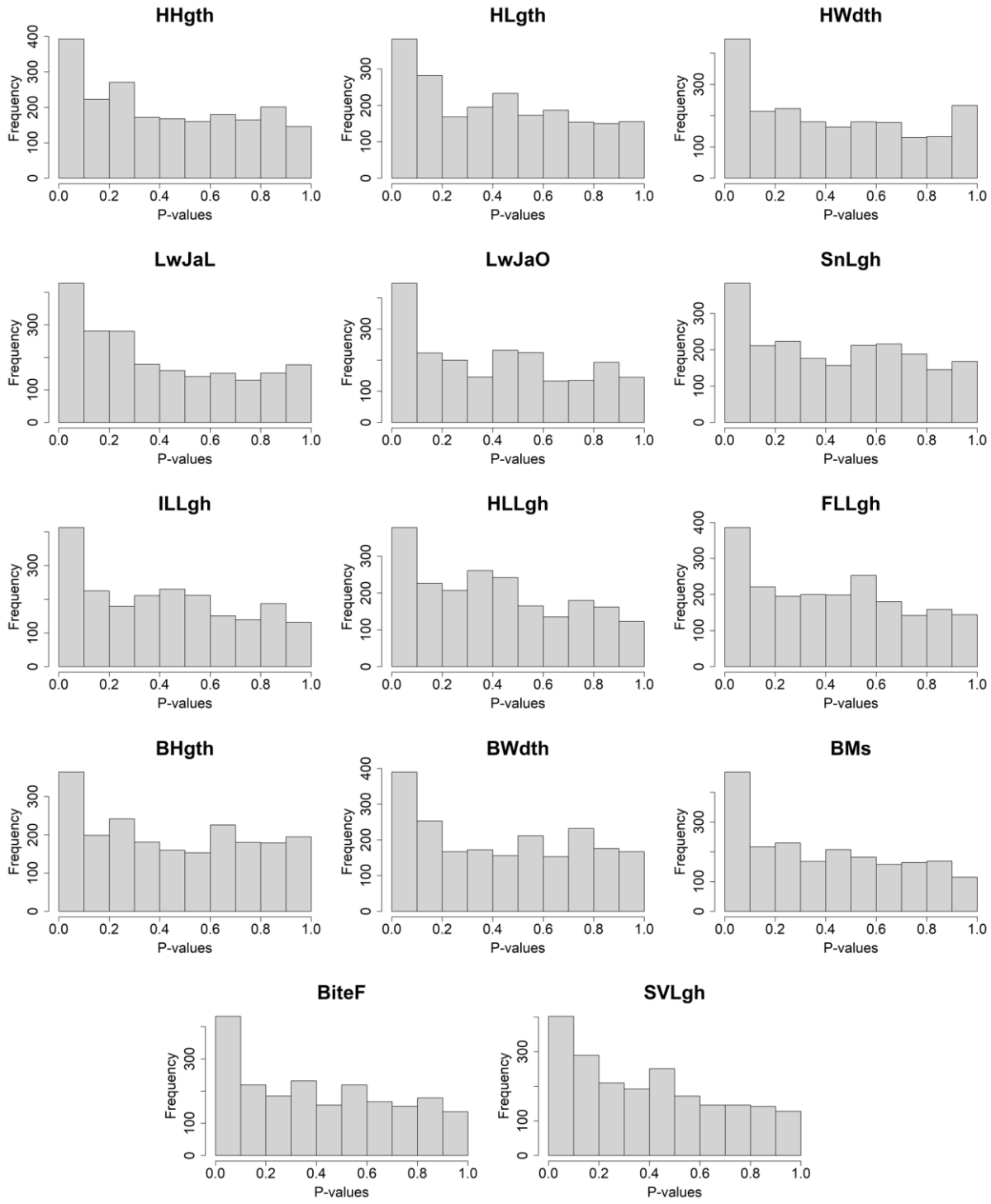

Figure S14 Histograms of adjusted p-values for all loci found in LFMM analysis of male *P. siculus* individuals from Pod Mrčaru and Pod Kopište islands. Phenotypic trait abbreviations are defined in Table 1.

8.2.6 Genotype-environment associations

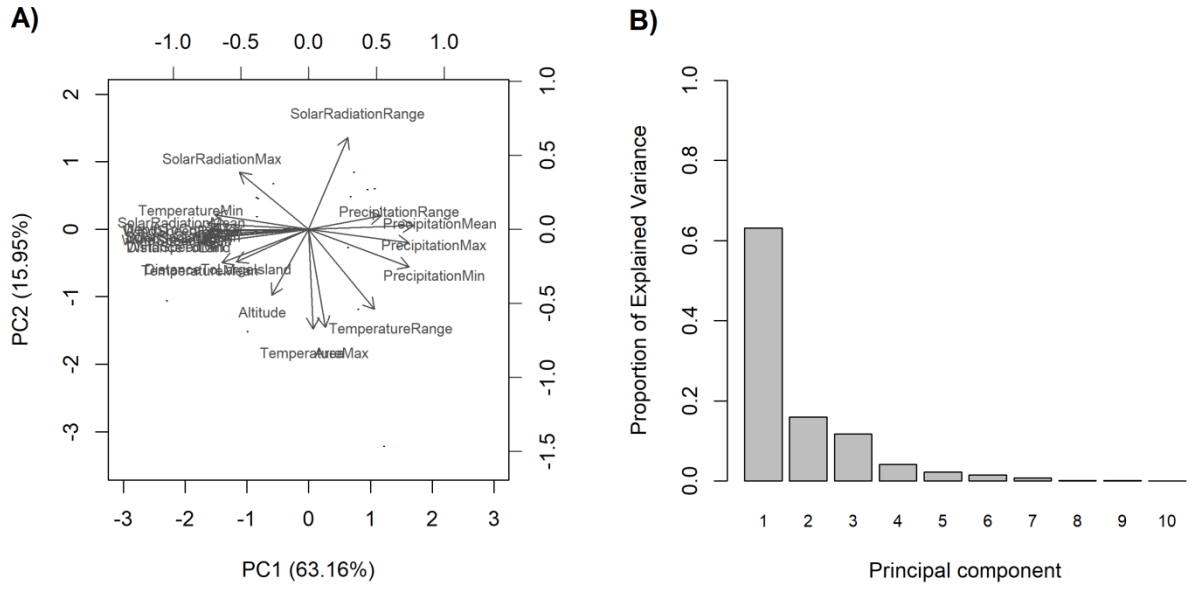

Figure S15 A) Biplot and **B)** proportion of variance explained by each principal component in the PCA analysis of ecological variables among all sampled *P. siculus* populations.

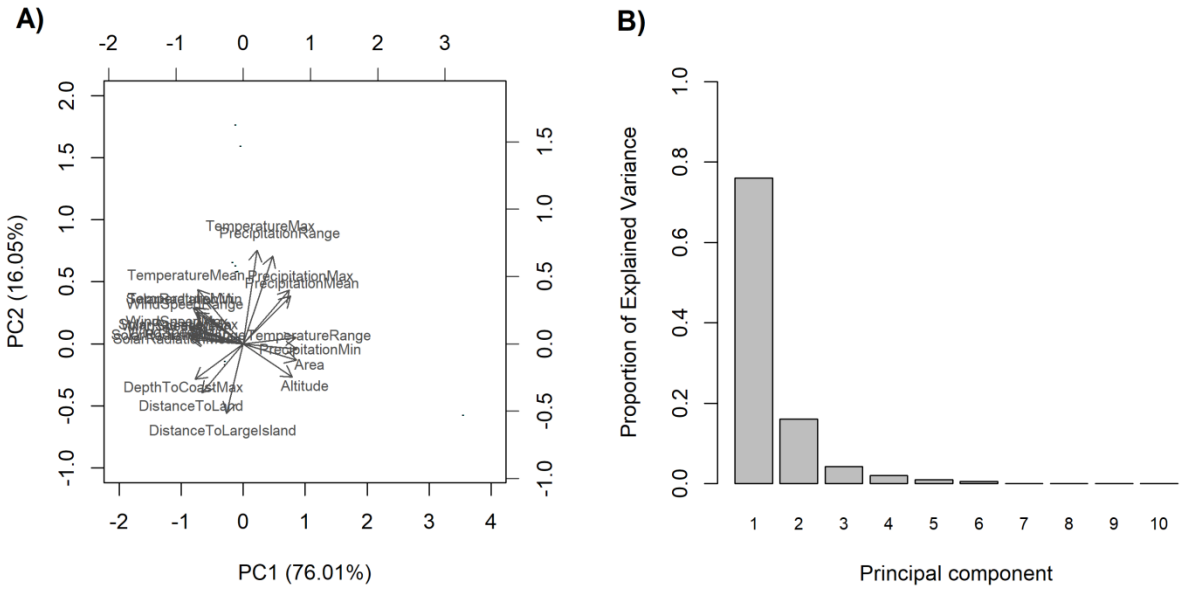

Figure S16 A) Biplot and **B)** proportion of variance explained by each principal component in the PCA analysis of ecological variables among all sampled *P. melisellensis* populations.

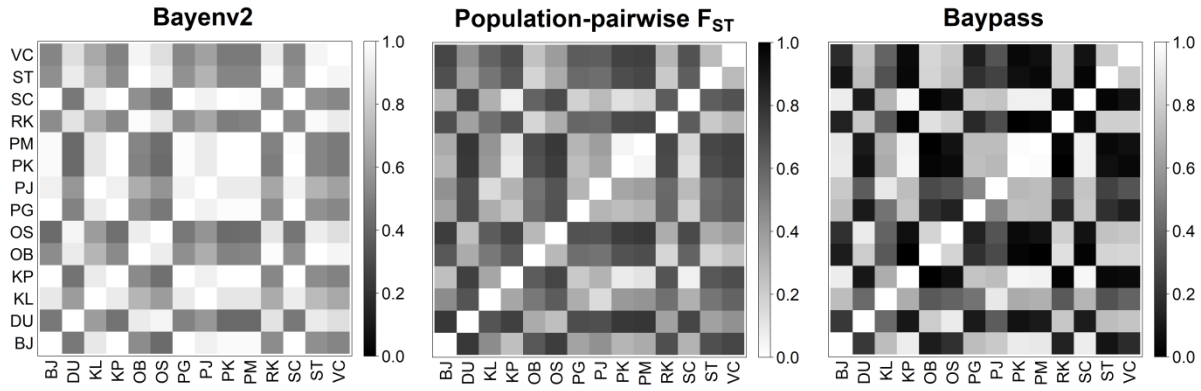

Figure S17 Population pairwise F_{ST} matrix similarity to covariance matrix obtained from Baynev2 (Mantel r = -0.96; p = 0.0001) and Baypass (Mantel r = -0.93; p = 0.0001) GEA analyses of 14 *P. siculus* populations. Population abbreviations are defined in Figure 5.

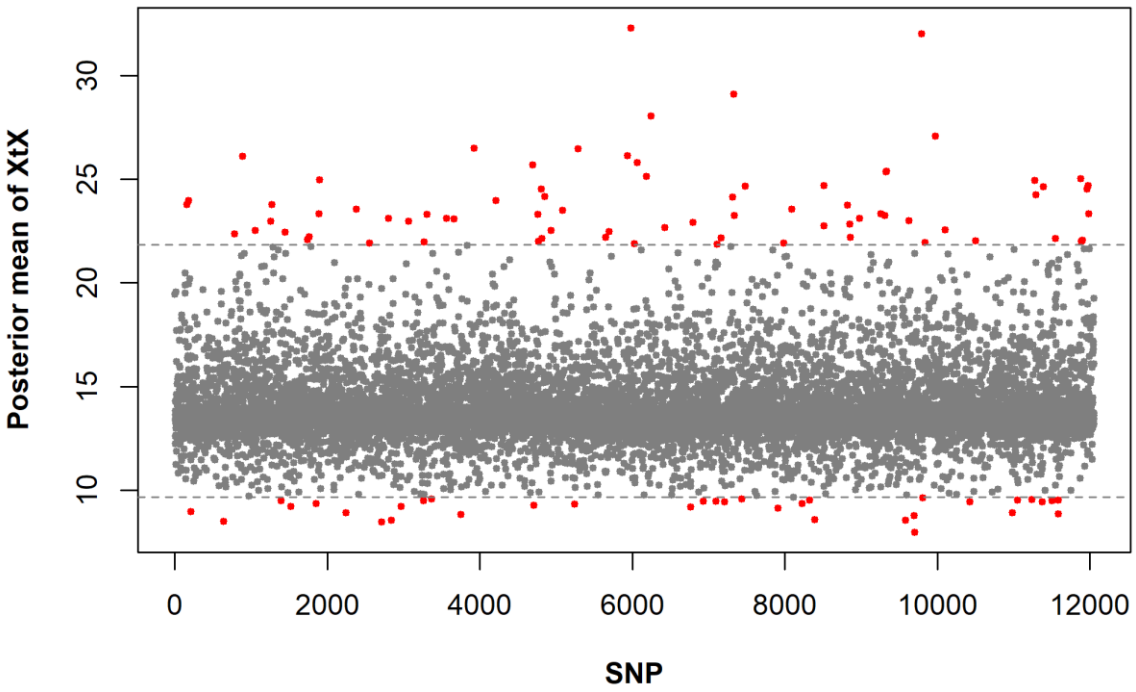

Figure S18 Manhattan plot of XtX values from Baypass core model analysis of 14 wild *P. siculus* populations. SNPs marked in red are considered to be under divergent (top) or balancing (bottom) selection. Dashed lines show 1% and 99% XtX values threshold obtained from Baypass core model analysis on simulated pseudo-observed datasets (PODs).

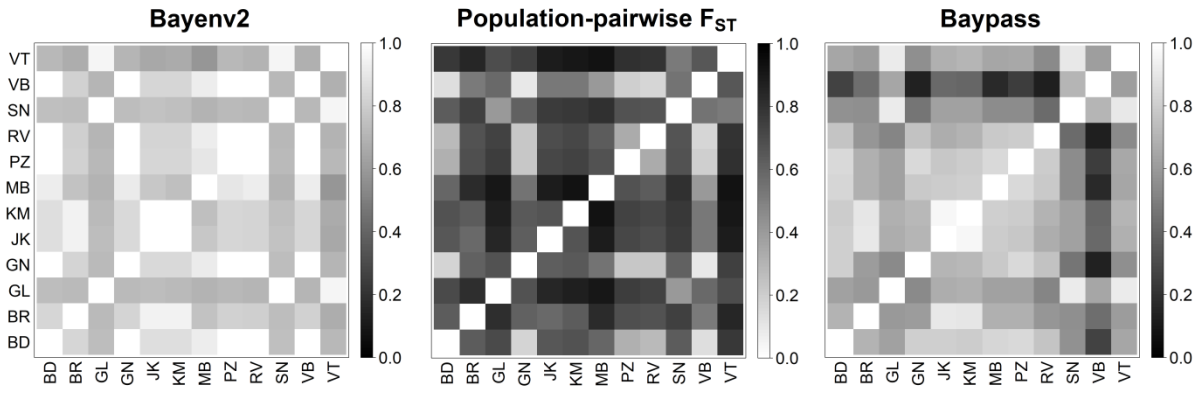

Figure S19 Population pairwise F_{ST} matrix similarity to covariance matrix obtained from Baynev2 (Mantel r = -0.8; p = 0.0002) and Baypass (Mantel r = 0.27; p = 0.9) GEA analyses of 12 *P. melisellensis* populations. Population abbreviations are defined in Figure 5.

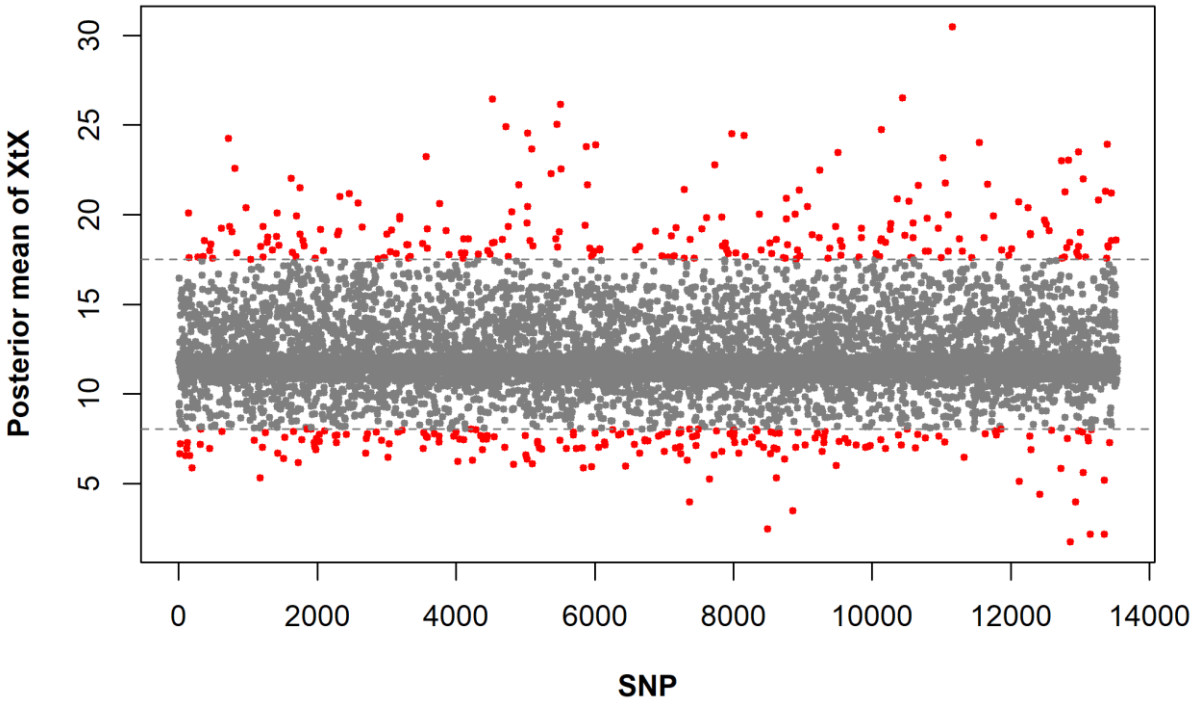

Figure S20 Manhattan plot of XtX values from Baypass core model analysis of 12 wild *P. melisellensis* populations. SNPs marked in red are considered to be under divergent (top) or balancing (bottom) selection. Dashed lines show 1% and 99% XtX values threshold obtained from Baypass core model analysis on simulated pseudo-observed datasets (PODs).

8.2.7 Phylogenetic analysis

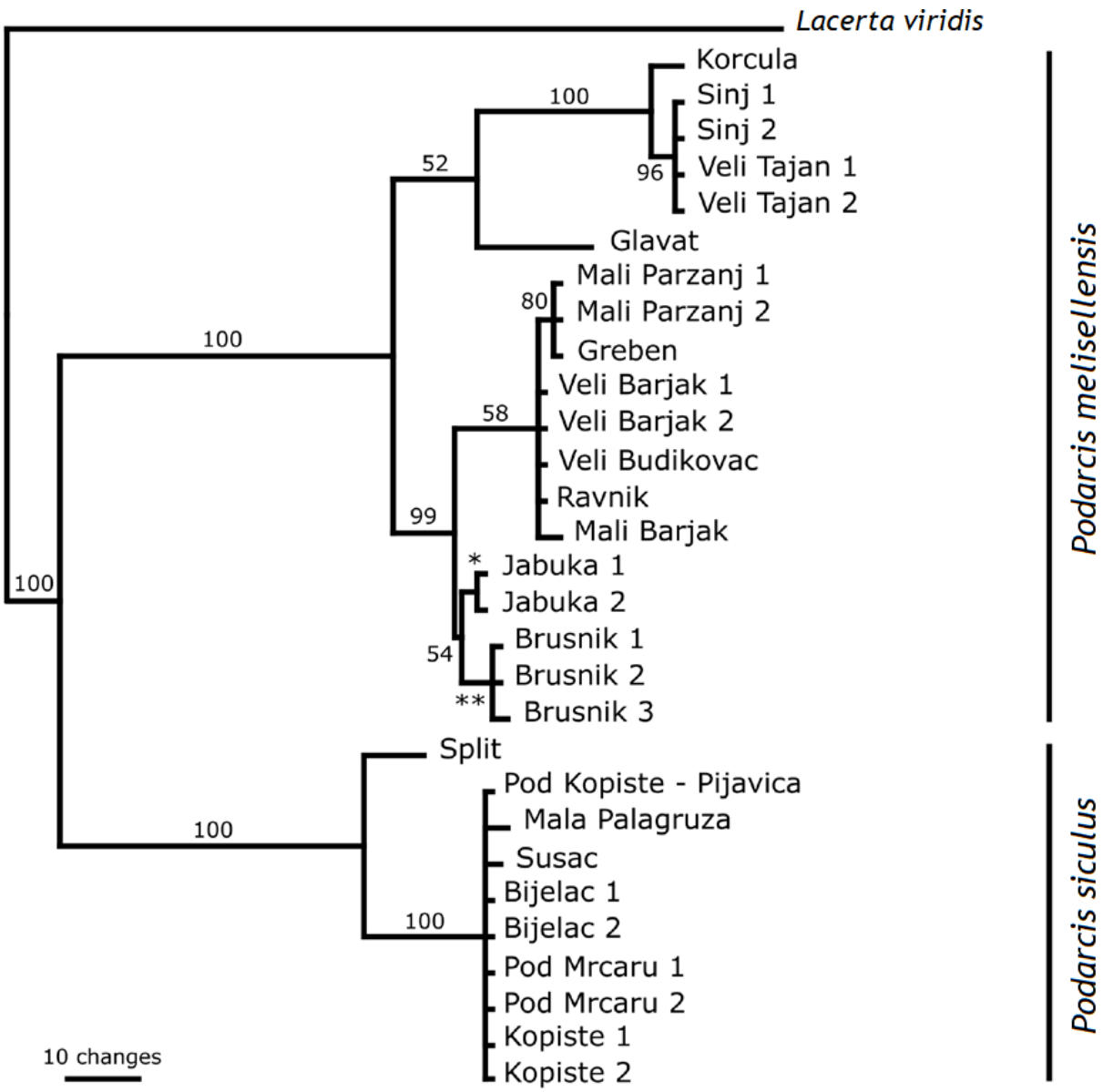

Figure S21 Phylogenetic relationships among 20 *Podarcis* populations and one outgroup, based on cytochrome *b* sequences and generated using maximum parsimony (MP). Branch lengths are proportional to the number of changes in the DNA sequences. Indicated bootstrap values for topology support are rounded (*96; **79).

9. CURRICULUM VITAE

Iva Sabolić was born on November 15th 1990 in Koprivnica, Croatia. She finished undergraduate studies in Environmental Sciences at Faculty of Science, University of Zagreb in 2012. She graduated with Masters in Ecology and Nature Preservation at Faculty of Science, University of Zagreb with the thesis ″Assessment of pollution exposure in *Mytilus galloprovincialis* Lamarck, 1819 using oxidative stress biomarkers″, in 2016.

From 2016 she works as a research and teaching assistant at Faculty of Science, University of Zagreb within InterregMed project ″Connectivity among Mediterranean fishery stakeholders and scientists resolves connectivity of fishery populations″, and HRZZ project ″Genomic aspects of rapid evolution of Italian wall lizard (*Podarcis sicula*)″. She was a teaching assistant in Invertebrates (2016), Zoology (2017, 2019), Evolutionary ecology (2018), and Laboratory skill training (2017, 2019) courses.

She was part of several international and domestic scientific conferences with oral and poster presentations, and attended a number of bioinformatics and population genomics workshops. Iva Sabolić is an author on three scientific papers (one of which is in the submission process):

Grbin D, Sabolić I, Klobučar G, Dennis SR, Šrut M, Bakarić R, Baković V, Radić Brkanac S, Nosil P, Štambuk A (2019) Biomarker response of mussel's regarding environmental conditions, pollution impact and seasonal effects; Science of The Total Environment, 694: 133470, doi: 10.1016/j.scitotenv.2019.07.276

Taverne M, King-Gillies N, Krajnović M, Lisičić D, Mira O., Petricioli D, Sabolić I, Štambuk A, Tadić Z, Vigliotti C, Wehrle B, Herrel A (2020) Proximate and ultimate drivers of variation in bite force in the insular lizards *Podarcis melisellensis* and *Podarcis sicula*, Biological Journal of the Linnean Society, 131: 88–108, doi: 10.1093/biolinnean/blaa091

Sabolić I, Baltazar-Soares M, Štambuk A (2020) Incorporating evolutionary based tools in cephalopod fisheries management, *under revision for Reviews in Fish Biology and Fisheries (RFBF-D-20-00047)*