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Vicariance divergence and gene flow among islet populations of an endemic lizard

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Abstract

Allopatry and allopatric speciation can arise through two different mechanisms: vicariance or colonization through dispersal. Distinguishing between these different allopatric mechanisms is difficult and one of the major challenges in biogeographical research. Here, we address whether allopatric isolation in an endemic island lizard is the result of vicariance or dispersal. We estimated the amount and direction of gene flow during the divergence of isolated islet populations and subspecies of the endemic Skyros wall lizard *Podarcis gaigeae*, a phenotypically variable species that inhabits a major island and small islets in the Greek archipelago. We applied isolation-with-migration models to estimate population divergence times, population sizes and gene flow between islet–mainland population pairs. Divergence times were significantly correlated with independently estimated geological divergence times. This correlation strongly supports a vicariance scenario where islet populations have sequentially become isolated from the major island. We did not find evidence for significant gene flow within *P. g. gaigeae*. However, gene-flow estimates from the islet to the mainland populations were positively affected by islet area and negatively by distance between the islet and mainland. We also found evidence for gene flow from one subspecies (*P. g. weigandi*) into another (*P. g. gaigeae*), but not in the other direction. Ongoing gene flow between the subspecies suggests that even in this geographically allopatric scenario with the sea posing a strong barrier to dispersal, divergence with some gene flow is still feasible.

Keywords: biogeography, dispersal, evolutionary divergence, gene flow, IMa2, vicariance

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Introduction

Both traditional views of speciation and recently emerging consensus strongly suggest that allopatric speciation is by far the most common mode of speciation (Coyne & Orr 2004; Price 2007). However, a classical source of controversy in biogeography and speciation research is the relative role of two different allopatric speciation scenarios: dispersal vs. vicariance (Brooks & McLennan 1991; Cowie & Holland 2006; Crisci & Katinas 2009). More specifically, are new species typically formed as an ancestral species colonizes novel areas (dispersal), or are novel and emerging geographical barriers more important (vicariance)?

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Historically, the distribution of taxa was thought to be driven by dispersal and taxa thought to disperse from centres of origin (Croizat *et al.* 1974; Bremer 1992; Crisci & Katinas 2009). Croizat *et al.*'s (1974) panbiogeography instead emphasized the importance of geological patterns and processes for the distribution of species, that is, a vicariance view. Later, cladistic biogeography (Nelson & Platnick 1981) merged the vicariance-based approach with cladistics to explain the distribution of different taxa (Crisci & Katinas 2009). Analyses applying molecular clocks mostly indicate that phylogenetic divergence is too recent to be explained by vicariance (see e.g. de Queiroz 2005). The evidence is, however, equivocal. While plant distribution is mainly explained by dispersal, animal distributions largely conform to geological vicariance (Sanmartin & Ronquist 2004). Vicariance is testable by the prediction

that the origin of a barrier should generate similar divergence times across multiple lineages of taxa (Crisp & Cook 2007). Although the discord between cladograms and fragmentation does not necessarily imply that dispersal is the predominant mechanism, it is in many cases the most plausible explanation when no such match is found (de Queiroz 2005). Comparisons of the divergence dates between populations with known geological histories are thus a promising method to distinguish between vicariance and dispersal scenarios. If vicariance is the predominant mechanism, a correlation of split times with historical geological events is expected. If fragmented areas are colonized after separation (i.e. a dispersal scenario), no such correlation is expected.

Clearly, the relative roles of geographical isolation, gene flow and selection in species divergence are of central theoretical importance to several issues in evolutionary biology, but are empirically extremely hard to resolve (Bolnick & Fitzpatrick 2007; Räsänen & Hendry 2008). Even moderate levels of gene flow have been shown to prevent divergence (Garcia-Ramos & Kirkpatrick 1997; Bolnick & Nosil 2007; Räsänen & Hendry 2008). To understand whether organisms in fragmented settings are mostly affected by historical contingencies or by influx of genes from other populations in metapopulation networks, studies of divergence times and gene flow are crucial. There are powerful population genetic analysis tools, which make it possible to date both splitting of populations and estimate gene flow between populations (see for example Hey 2010a). Such studies are difficult to perform, and this research area is still subject to some controversy (Cowie & Holland 2006; Heaney 2007; Schilthuizen *et al.* 2010; Strasburg & Rieseberg 2011).

Here, we employ an integrative approach with both a simulation study based on molecular divergence and a coarse-grained analysis of geological divergence to an island–mainland system of lizards (genus *Podarcis*) in the Aegean archipelago. We investigate the roles of vicariance and dispersal and gene flow in population divergence in this system. Greece and the Aegean archipelago is a hotspot for reptile diversity with very high levels of endemism. There are six endemic lacertiid lizard species in Greece, from which five are island endemics (Arnold & Ovenden 2002; Lymberakis *et al.* 2008). The biogeography and history of the Aegean Sea with continental landbridges separated into islands by raising sea levels during the Holocene (Lambeck 1996) provides an ideal setting for the study of how geographical isolation affects population genetics (Hurston *et al.* 2009). There are studies on lizards in the Mediterranean, which support a vicariance divergence scenario (Castilla *et al.* 1998) and a dispersal scenario (Carranza *et al.* 2006; Brown *et al.* 2008; Paulo *et al.* 2008). There are also several documented extinction events (Foufopoulos *et al.* 2010), and if such extinctions are prevalent, dispersal and re-colonization would be important in moulding islet populations. The occurence of tsunamis in the eastern part of the Mediterranean (Papadopoulos *et al.* 2010) and the farming habit of periodical burning of thorny vegetation following over-grazing in the Greek archipelago (Geeson *et al.* 2002) provide two potential mechanisms that could cause such local extinctions on small islets in the Aegean Sea. We use isolation-with-migration models to study the mechanisms driving population divergence in islet populations of the Skyros wall lizard (*Podarcis gaigeae*). In particular, we address the question whether vicariance or dispersal has been the main force in the establishment of populations on the islets in the Skyros archipelago. We also discuss how the biogeographical setting has affected population sizes over time and levels of gene flow between populations.

Materials and methods

Study species and geographical setting

The Skyros wall lizard *Podarcis g. gaigeae* is a small, lacertiid lizard endemic to the island of Skyros, Greece, and its surrounding archipelago of islets. The subspecies *Podarcis g. weigandi* is found on the island of Piperi, situated 40 km north of Skyros (Fig. 1). Islet populations of the Skyros wall lizard are morphologically strongly diverged from the population on the main island of Skyros. Several instances of island gigantism have been reported (Pafilis *et al.* 2009a,b; A. Runemark and E.I. Svensson unpublished data), and the islet populations also differ strongly in throat colour morph frequencies (Runemark *et al.* 2010). We studied seven of these morphologically very strongly diverged small islet populations (Pafilis *et al.* 2009a; A. Runemark *et al.* unpublished data) and also included data from a northern subspecies (*P. g. weigandi*) on the island of Piperi (Fig. 1).

Owing to the sea level rise in the Aegean Sea during the last 18 000 years (Lambeck 1996), the islets in the Skyros archipelago became isolated from the main island of Skyros. The time of isolation of the islets is proportional to the sea depth between the islet and the main island of Skyros (Lambeck 1996; Perissoratis & Conispoliatis 2003; Hurston *et al.* 2009). We estimated the geological divergence time from a 1:25 000 bathymetric map of the island of Skyros and its surrounding archipelago (available from the Hellenic Navy Hydrographic Service; http://www.hnhs.gr/portal/page/portal/HNHS) by locating the shallowest passage between the respective islet and the main island of

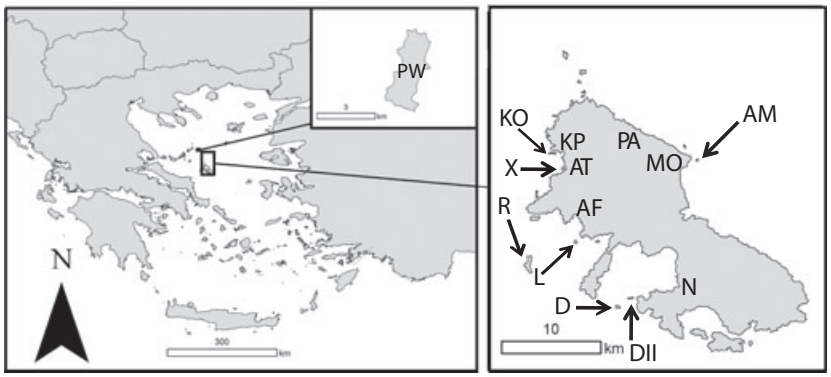

Fig. 1 Map of Greece and the Aegean archipelago with magnifications of the two main islands Skyros (lower right) and Piperi (upper right). The letter codes denote sampling localities. Islet localities are denoted with both letter codes and arrows.

Skyros. These depths were then located in the figures of detailed records of sea level change the Aegean (Lambeck 1996) and world sea level (Fairbanks 1989) in Adobe Illustrator CS 3. The divergence dates, corresponding to the time when the most shallow passage points between the islets and the main island of Skyros were above sea level, were used as geological divergence dates in the models in this paper, following Hurston *et al.* (2009). To evaluate whether the geological divergence times from the different sea level records were consistent, correlations were calculated between the different estimates.

Field work and genetic sampling

Lizards were caught on seven islets close to the coast of Skyros and the closest suitable localities on main Skyros (hereafter referred to as 'mainland populations') to obtain a geographically replicated study design with multiple islet–mainland pairs (Fig. 1A). We assume that these mainland populations are the most probable founding populations and that the migrants to the islets come from the closest mainland populations. Although the mainland 'populations' might not be separate populations in the strict sense (see e.g. Waples & Gaggiotti 2006), we use this term to describe lizards caught at a specific geographical mainland location. Mainland populations have a low degree of population differentiation (see F_{ST}-values, Table S1, Supporting information) and show only weak isolation by distance (Runemark *et al.* 2010), indicating that there are no strong population structures or main barriers to gene flow on the main island of Skyros. At least 27 lizards from each sampled populations were caught in the field between 2007 and 2009; *P. g. weigandi* were caught on the island of Piperi in 2008. For a summary of number of sampled individuals, see Table 1. A small piece of tail sampled for DNA analysis was preserved in 96% ethanol.

DNA extraction and microsatellite typing

DNA from tail samples was extracted with an ammonium acetate extraction protocol (Richardson *et al.* 2001). The concentration of the extracted DNA was quantified in a Bechman DU64 spectrophotometer (Bechman Coulter, Inc., Brea, CA, USA), and samples were diluted to 10 ng/μL and used as templates in PCR using primers from the study described by Runemark *et al.* (2008) and Wellenreuther *et al.* (2009). PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems Inc., Foster City, CA, USA), and the conditions used were as specified in Runemark *et al.* (2008) and Wellenreuther *et al.* (2009), respectively. The PCR products were separated and alleles were detected in an ABI PRISM 3730 capillary sequencer (Applied Biosystems). GeneMapper (Applied Biosystems) was used to determine the genotypes of individuals. Microsatellites were checked for the presence of null alleles using Micro-Checker, a program that has the advantage that it estimates both heterozygote deficiency and long-allele dropouts (Van Oosterhout *et al.* 2004). An F_{ST}-outlier analysis (Beaumont & Nichols 1996) implemented in Lositan (Antao *et al.* 2008) was applied to identify and exclude microsatellite loci that were F_{ST}-outliers and therefore potentially under selection. fstat version 2.9.3 (Goudet 2001) was used to investigate whether loci departed significantly from Hardy–Weinberg equilibrium and to calculate pairwise F_{ST}-values. To illustrate the degree of divergence between populations, the programs Structure (Pritchard *et al.* 2000) and CLUMPP (Jakobsson & Rosenberg 2007) were used. Ten repetitions of the Structure runs, each with a burn-in period of 20 000 and 50 000 permutations, were performed for all K between 1 and 21. A method developed by Evanno *et al.* (2005) was used to identify the most probable K, and this K was then illustrated with a figure created in CLUMPP.

Table 1 Number of genetic specimens per population, islet area, distance to the main island of Skyros, and depth of the shallowest passage between islet and main island of Skyros from the 1:25 000 bathymetric map of the island of Skyros and its surrounding archipelago (available from the Hellenic Navy Hydrographic Service; http://www.hnhs.gr/)

Population	Habitat	Samples	Log islet area	Distance to mainland (m)	Shallowest depth (m)	Divergence time (Lambeck 1996)	Divergence time (Fairbanks 1989)
AgiarMolau (AM)	Islet	34	3.98	71	4	5700	1500
Molos (MO)	Mainland	34	8.32	NA	NA	NA	NA
ExoDiavates (DII)	Islet	27	4.62	359	10	6500	6000
Mesa Diavates (D)	Islet	102	4.41	1463	34	8700	8700
Nyfi (N)	Mainland	148	8.32	NA	NA	NA	NA
Kotslee (KO)	Islet	36	4.98	237	15	7000	7000
KyriaPanagia (KP)	Mainland	34	8.32	NA	NA	NA	NA
Lakonissi (L)	Islet	156	4.33	710	52	9700	9500
AgiosFokas (AF)	Mainland	69	8.32	NA	NA	NA	NA
Erinia (R)	Islet	32	5.79	4453	174	18 000	18 000
Island of Atsitsa (X)	Islet	37	4.65	132	5	5800	2500
Atsitsa (AT)	Mainland	60	8.32	NA	NA	NA	NA
Palomares (PA)	Mainland	46	8.32	NA	NA	NA	NA
Podarcis g. weigandi (PW)	Piperi	34	—	42 000	—	—	—

Divergence time estimates based on the depths of the shallowest passages are estimated for historic sea level estimates for the Aegean Sea from Lambeck (1996) and for the world from Fairbanks (1989), respectively.

Gene-flow analyses—priors, assumptions and computations

The program IMa2 (Hey 2010a) was used to estimate gene flow between populations. The program is based on an isolation-with-migration (IM) model and uses Metropolis-coupled Markov chain techniques to estimate the posterior densities of the time of divergence, population size and gene flow (Nielsen & Wakeley 2001; Hey & Nielsen 2007; Hey 2010a). The model assumes random population samples, a neutral mutation model (Kimura 1983), freely recombining loci and constant population sizes and gene exchange rates. Although modelling constant population sizes in a scenario with rising sea levels and shrinking habitable area might not be ideal, modelling general patterns requires simplifications and this is presently the only option in programs such as IMa2 (Hey 2010a) and Migrate (Beerli & Felsenstein 2001).

We excluded loci containing imperfect repeats and used the stepwise mutation model in IMa2 to analyse the data (Ohta & Kimura 1973; Hey 2010a). The analyses were based on 11 highly variable microsatellite loci: Lv319, B4, Lv472, Pod1B, B6, Pb73, Po47, Po56, Po55, Po22 and Po43. The IMa2 program requires the use of uniform priors for divergence times and population sizes. For migration rates, exponential priors can be more informative when prior information suggests that actual rates of gene flow are very low or zero. As we have no prior information on gene-flow rates for the Skyros wall lizard and quite high levels of gene flow could be feasible given the short geographical distances, the suggested rafting capabilities of related lizards (Paulo *et al.* 2008) and the gradual isolation of the landmasses, uniform priors were used also for migration rates. The demographic parameters of the isolation-with-migration model, as implemented in IMa2 and related programs, are all scaled by the mutation rate (Hey 2010a; Nielsen & Wakeley 2001). The mutation rate is not a part of the Bayesian analyses of the isolation-with-migration model; however, the user must provide one in order to convert parameter estimates to more interpretable demographic units. Two alternative calibration points for mutation rates of the microsatellite loci were available, one for the sister species *Podarcis milensis* and one for the subspecies *P. g. weigandi*. Goldstein *et al.*'s (1995) δu^2, where δ is defined as the average squared difference in repeat numbers for two alleles drawn one from each different population for two populations isolated t generations in the past, and u is the mutation rate (mutations/locus/generation), was used to estimate the mutation rate. For *P. milensis* and *P. g. weigandi*, δ was derived from microsatellite data. For *P. milensis*, δ was divided with a published estimate of the mtDNA sequence divergence time between *P. milensis* and *P. g. gaigeae* (Poulakakis *et al.* 2005), and for *P. g. weigandi*, δ was divided by a geological splitting time estimate (Dermitzakis & Papanikolaou 1981; Perissoratis & Conispoliatis 2003) to obtain u^2. The fit between the calibration with *P. milensis* gave a mean per-generation mutation rate of $u = 0.001$, and the calibration with *P. g. weigandi* gave a mean

mutation rate of $u = 0.00001$. An intermediate mutation rate value of $u = 0.0001$ was therefore applied to all the analyses in this study.

The upper limit of the prior for divergence time was set to $t = 1$. To place this in a demographic context, with a mutation rate of $u = 0.0001$, and assuming a 2 year generation time, this value corresponds to a demographic splitting time ($T = t \times g/u$) of 20 000 years. By comparison, the islets in the Aegean have become isolated because of the sea level rise that has taken place during the last 18 000 years (Lambeck 1996; Hurston *et al.* 2009). The geometric mean of δu^2 estimates of $4Nu$ (Goldstein *et al.* 1995), where δ is defined as the average squared difference in repeat numbers for two alleles from the same population, u is the mutation rate and N is the population size, within *P. g. gaigeae* is 2.5. This leads us to consider a prior distribution of $4Nu$ with an upper bound of 15. However, this value was found to be too low to include the entire probability density distribution for some ancestral population sizes (data not shown), and based on the shapes of the posterior distributions from our initial runs, we therefore increased the upper bound of the prior for $4Nu$ to 30. The lizard density is quite high at some localities (Pafilis *et al.* 2010), and large ancestral population sizes can be expected because of the larger land area before inundation. The upper bound of the migration priors were set to $m = M/u = 0.5$, where M is the migration rate per gene copy per generation. These upper bounds for $4Nu$ and M/u together can accommodate values for the population migration rate (i.e. $2NM = 4Nu \times M/u/2$) well above one. Because $2NM$ is a function of two parameters and is not a parameter itself, the upper bound on $2NM$ that effectively ends up being accommodated by the model depends on the parameter estimates. For example, if the δu^2-based estimate of $4Nu$ (i.e. 2.5) is accurate, then an upper bound on M/u of 0.5 would effectively place an upper bound on $2NM$ of $0.5 \times 2.5/2 = 0.625$. While this is less than the threshold value of 1, at which point gene flow begins to create the appearance of a single population (Wright 1931), it should still be sufficiently high to detect moderate levels of gene flow.

A generation time of 2 years was used because of the presence of a distinct class of sub-adults in the populations even before the first-clutch hatches in the spring, and females from this size and morphology class do not have mating scars (A. Runemark, personal communication). As the IMa2 program is slow for large microsatellite data sets, 50 gene copies were randomly selected from each population and used in the analyses.

Initial runs with 50 gene copies, a burn-in period of 20 000 and a run length of 100 000 were performed to assess whether the priors were suitable and the heating conditions were appropriate for the data set. To ensure adequate mixing of the Markov chain, we used Metropolis-coupling of 70 independent heated chains (Geyer 1991). To ensure that the Markov chains mixed sufficiently, two long independent runs with 36 000–157 000 chains were performed for each analysis. Stationarity was reached already during the burn-in period. Within-run stationarity was assessed by inspection of autocorrelations of splitting time terms, by comparisons between parameter estimates generated from genealogies from the first and the second half of the run, respectively, and by visual inspection of the splitting time trend plots to assure that no trends were present (Hey 2010b). Burn-in duration was set to 300 000 steps, and the most heated chain had a heating factor of 0.9, with other chains having heating values between 1 (i.e. no heating) and 0.9. The final analysis was based on genealogies sampled from two independent runs. For each islet, runs were carried out both with the closest mainland populations and with gene copies sampled randomly from all sampled mainland populations.

Estimates of migration rates, time of divergence and population sizes

The rate at which one population received migrants from another, $2NM$, was derived from integrating over the joint posterior density for the population size and migration parameters (Hey 2010a). The migration rate per gene copy per generation (M) was estimated through multiplying the migration estimate m by the mutation rate u ($M = m/u$). To test whether there is a significant population migration between populations, a test developed by Nielsen & Wakeley (2001) implemented in the program IMa2 (Hey 2010a) was applied. The divergence time parameter, $t = Tu$, was converted into years by dividing with the assumed mutation rate $u = 0.0001$ and multiplying with the generation time. The population size (N) was estimated from the population size parameter estimate (q) by dividing with $4u$ ($N = q/4u$).

Analyses of factors affecting divergence time and gene-flow levels

We performed a regression analysis between the geological divergence times (derived from depth and the sea level record for the world sea level; independent variable) and the molecular divergence times (dependent variable) to investigate whether molecular divergence was affected by the time when the islets became isolated. Although molecular divergence can precede population splitting, this is improbable to have happened in the case of the dense main island Skyros wall lizard population that has low levels of genetic

differentiation across the entire main island of Skyros (Runemark *et al.* 2010). In this regression model, we did thus not include an intercept, because this would have no obvious biological meaning (i.e. a positive/negative intercept would indicate that molecular divergence started before/after geological divergence). To confirm that this exclusion of the intercept does not alter the results, we performed the same analysis with an intercept.

To investigate which factors affect the population migration rates, we analysed the highest points of the posterior distribution of this variable using a GLM approach in Statistica (Statsoft 2004). The distance to the main island of Skyros and the area of the islet were covariates in the model, and we also included the two-way interaction between them. The analysis was performed for both mainland-to-islet migration and islet-to-mainland migration. Nonsignificant variables were sequentially removed by backward deletion, starting with the interactions. Sign tests were used to test for asymmetry between mainland and islet localities in the effective number of migrating gene copies per generation and migration rate per gene copy, respectively. Sign tests were used because the migration prior puts an upper limit to migration levels, and estimates can thus be truncated. The subspecies *P. g. weigandi* was a priori not included in the statistical analyses because we are addressing within-subspecies divergence. In addition, we have no geological divergence time estimate for Piperi because the depth exceeds that of the lowest sea level and it is thus not possible to estimate the divergence time using the same method as described above.

Results

Geological separation times for when the islets became isolated from the mainland were estimated based on Fairbanks (1989) sea level records, which ranged between 1500 and 18 000 years, and Lambeck (1996), which ranged from 5700 to 18 000 years (Table S2). The two estimates were highly significantly correlated ($F_{1,5} = 57.87$, $P = 0.0006$, $R^2 = 0.53$). The discrepancy with the lowest date is possibly a result of the slow increase in sea level over the last 6000 years (Fairbanks 1989; Lambeck 1996), which makes small differences in depth resulting in large differences in time of divergence, and the fact that Lambeck (1996) did account for tectonic uplift based on specific regions of the Aegean Sea.

Evaluation of the population structure using the program Structure (Pritchard *et al.* 2000) and applying the Evanno *et al.* (2005) method indicate 12 genetic groups in the data set (Fig. 2; see also Table S1, Supporting information). Generally, the use of gene copies from the pooled mainland population gave similar results in the IM models (data not shown) as when gene copies were sampled from only a single mainland population (i.e. the closest sampled mainland population; Fig. 1). However, for the islets that have been separated for a longer period of time (e.g. PW, the subspecies *Podarcis g. weigandi* on the island of Piperi, and *R*, the population on the islet Erinia furthest from mainland Skyros in the archipelago), the results were not consistent. Comparison with the available background information (i.e. the geological divergence times) suggested that the analyses based on gene copies sampled from the closest population gave more accurate estimates and the runs also reached stationarity faster with this data set. We therefore use and present the results from the runs between an islet and its closest mainland population.

Divergence times were estimated to range between 250 and 2790 years for the islet populations of *Podarcis g. gaigeae*, and the subspecies *P. g. weigandi* was estimated to have become isolated *c.* 17 270 years ago, that is, just after sea level rise started about 18 000 years ago

Table 2 Estimated divergence time, population sizes and population migration rates for all IMa2 models

Populations	Divergence time (years)	Pop. size islet	Pop. size mainland	Pop. size ancestral	Migration M to I	Migration I to M
AM—MO	450	207	994	15 850	0.0011	0.0180
DII—N	1950	1182	5457	17 163	0.1376	0.0037
D—N	2210	1294	7407	15 063	0.1665	0.0037
KO—KP	250	207	10 557	15 700	0.0003	0.2661
L—AF	1970	394	3394	17 875	0.0498	0.0016
R—AF	2790	1744	4144	18700	0.0668	0.0023
X—AT	1010	732	2682	16 000	0.0435	0.1162
PW—PA	17 270	3244	10 744	37 475	0.0003	0.1512*

We assumed a mutation rate of 0.0001. Migration denotes the population migration rate 2*NM*, and M to I is an abbreviation for mainland to islet and I to M for islet to mainland. Significance at the P < 0.05 level is denoted by *.

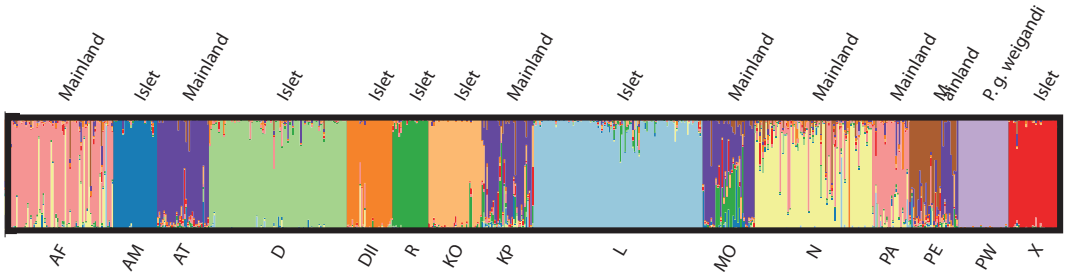

Fig. 2 Population structure of the Skyros wall lizard derived from Structure analyses show considerable population structure in the Skyros wall lizard. Labels above the figure denote whether the populations are mainland or islet populations or belong to the subspecies *Podarcis g. weigandi*. The population letter codes corresponding to Fig. 1 are found below.

(Fairbanks 1989; Lambeck 1996; Fig. 3; Table 2). Population size estimates were lower for the islets, where they ranged between 207 and 1744, while mainland population sizes varied from 994 to 10 557. *P. g. weigandi* had an estimated population size of 10 744. Generally, estimates of the ancestral population sizes were larger (ranging from *c.* 15 000 to 37 500 individuals; Table 2) than the current population sizes, which is in accordance with a bigger landmass that was subsequently inundated by rising sea levels. Divergence times, ancestral population sizes and current population sizes are schematically summarized and illustrated in Fig. 4.

In Fig. 5, we have plotted the posterior densities for population migration rates (A,B) and migration rates per gene copy per generation (C,D) for all mainland–islet population combinations; the subspecies *P. g. weigandi* is depicted in red. Three of the eight curves have nonzero peaks for migration for population migration rate from islets to mainland (A), whereas two of the eight curves have nonzero peaks for migration for population migration rate from mainland to islets (B). For *m*, migration rate per gene copy per generation, the results are only clearly defined for *P. g. weigandi*, which does show a significant gene flow into *P. g. gaigeae* (C) but does not have any significant influx of genes from *P. g. gaigeae* (D). A tendency for higher probability density at zero migration from islets to mainland than vice versa can be seen when comparing C to D.

As the posterior densities for most gene-flow rates are quite flat, it is likely that a much higher migration prior might have yielded results that converged on an island model where divergence time and gene flow could not be disentangled. It is known that for histories and data like those studied here, for recent divergence where there is limited information in the data on gene flow, that a high upper bound on the migration rate can drive the analysis towards an equilibrium island model (Hey 2010b, see also IMa2 Manual http://lifesci.rutgers.edu/~heylab/ProgramsandData/Programs/IMa2/Using_IMa2_10_13_10.pdf). In fact, the upper bound on the prior for gene flow, $m = 0.5$, was reached in six of eight cases of the migration rate per gene copy per generation from mainland to islet as well as for three of eight cases from islet to mainland. In the case of the population migration ($2NM$) rates, estimates varied between 0.002 and 0.27 from islet to mainland and between 0.0003 and 0.17 from mainland to islet, respectively (Table 2). The only statistically significant population migration detected by the Nielsen & Wakeley (2001) test was that from the Palamari mainland population on Skyros into the subspecies *P. g. weigandi*, which was significant at the $P < 0.05$ level.

The estimated molecular divergence times were significantly correlated with the geological divergence time estimated from Lambeck's (1996) sea level estimates in a regression model with the intercept fixed at zero ($F_{1,6} = 44.37$, $P = 0.0005$, molecular divergence = 0.17 × geological divergence, $\beta = 0.94 \pm 0.14$; $R^2 = 0.53$; Fig. 3b). The estimated molecular divergence time was thus on average a factor 5.8 lower than the geological one. This suggests that the mutation rate we initially assumed was too low. Multiplying the assumed mutation rate by 5.8 would make the scales of the molecular and geologically estimated divergence times show a closer fit. The corresponding regression was also highly significant if Fairbanks (1989) sea level data were used ($F_{1,6} = 40.24$, $P = 0.0007$, molecular divergence = 0.18 × geological divergence, $\beta = 0.93 \pm 0.15$; $R^2 = 0.53$), and in this case, the molecularly estimated divergence time was *c.* 5.6 times lower than the geological divergence time. This regression was also significant if an intercept was included ($F_{1,5} = 7.10$; $P = 0.045$, molecular divergence = 500 + 0.13 × geological divergence, $\beta = 0.77 \pm 0.29$; $R^2 = 0.59$).

Neither islet area, distance to the main island nor islet area × distance to the main island significantly explained population migration rate (number of migrant gene copies per generation) from mainland to islets (all $P > 0.17$). From islet to mainland, we found that the interaction between distance to mainland and islet area was not significant ($F_{1,3} = 0.083$, $P = 0.79$), and

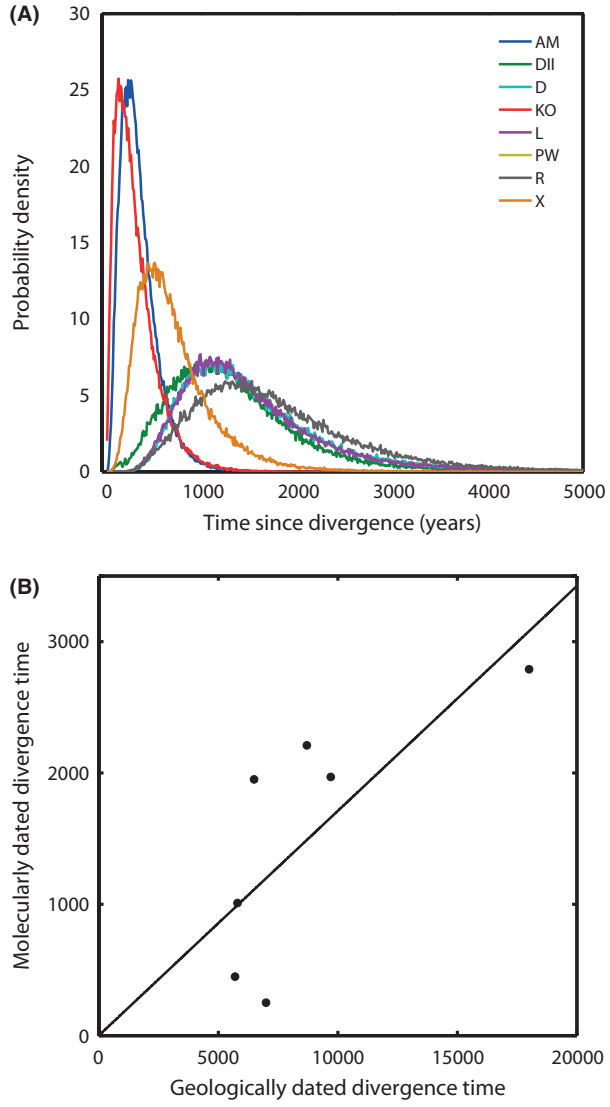

Fig. 3 (A) Probability distributions of molecular divergence times for the different islets. The letter abbreviations are marked on respective islets in Fig. 1. Because of the much longer divergence time driven by a different geological process, the subspecies *Podarcis g. weigandi* on the island of Piperi is not represented in the graph. (B) Molecularly estimated divergence time as function of Lambeck (1996) geologically estimated divergence time [pairwise comparisons between 'Mainland' (island of Skyros) and the various islets]. There is a significant positive correlation between the divergence times derived from sea depth and sea level change over time and divergence times derived from the isolation-with-migration model ($F_{1,6} = 44.37$, $P = 0.0005$, $\beta = 0.94 \pm 0.14$). Note that we used a regression model with no intercept (see Materials and methods for details). The relationship between the divergence times is given by the equation genetic divergence time = geological divergence time × 0.17.

this interaction was therefore removed from the model. In the final (reduced) model, population migration rate was negatively affected by distance to mainland ($F_{1,3} = 9.03$, $P = 0.040$) and positively by islet area ($F_{1,3} = 7.78$, $P = 0.049$).

We found no evidence for asymmetries in direction (mainland to islet vs. islet to mainland) for neither gene flow (sign test, $Z_4 = 0.50$, $P = 0.62$) nor migration rate per gene copy per generation (sign test, $Z_4 = -0.35$, $P = 0.72$). Although this suggests that there is no evidence of asymmetric migration, the low prior on migration (e.g. the mean of the maximum value of $2NM$ of 0.625) for the islet populations with small effective population sizes could have constrained higher levels of migration and concealed real and existing asymmetries. For the larger mainland populations, no such constraint of the migration prior is expected owing to larger effective population sizes.

Discussion

The islet populations of the Skyros wall lizard have relatively recently become isolated because of rising sea levels. The geological divergence times based on two different sea level estimates ranged from 1500 to 9500 years ago (Table 2). There is a highly significant correlation between the molecular divergence times and the geological divergence times (Fig. 3b), and this correlation is not affected by scaling the mutation rate. Because estimates of geological divergence explain over 50% of the variance in molecular divergence, the data support a vicariance model in which the islet populations have sequentially become separated from each other, coinciding with the formation of a barrier to dispersal (in this case: the sea) (Crisp *et al.* 2011). If extinctions and recolonization were prevalent, we would not expect to find such a pattern (de Queiroz 2005). Hence, this correlation suggests that the populations currently inhabiting these islands are relict populations of a larger ancestral population that included both the island of Skyros and all the current islet populations (Fig. 1). Furthermore, this correlation also strongly suggests that the molecular markers and the isolation-with-migration models applied are appropriate and accurate descriptors of this system.

In previous studies of lizards, there is both studies supporting vicariance-driven divergence (Jordan & Snell 2008; Hurston *et al.* 2009) and dispersal-driven divergence (Raxworthy *et al.* 2002; Calsbeek & Smith 2003; Amer & Kumazawa 2008; Paulo *et al.* 2008), and cases where a mixture of the mechanisms is most probable (Arntzen & Sa-Sousa 2007). Dispersal has been shown to be an important mechanism for divergence even on a large scale in reptiles (Raxworthy *et al.* 2002; Calsbeek & Smith 2003) and the prevalent mechanisms in some other lizard species in the Mediterranean (Carranza *et al.* 2006; Brown *et al.* 2008). In addition, molecular evidence suggests that a *Podarcis* lizard has crossed

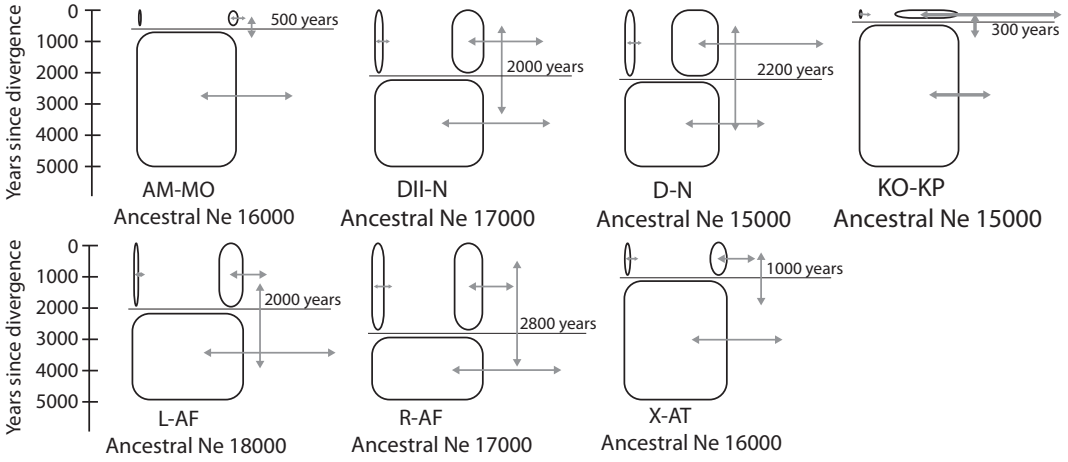

Fig. 4 Estimated population historical scenarios, including ancestral population sizes and divergence times for all seven islet–mainland population pairs of *Podarcis g. gaigeae*. Population assignations are found under the respective figures (see also Fig. 1 for geographical locations). The subspecies *Podarcis g. weigandi* is not represented here because of an estimated divergence time that was an order of magnitude larger. None of the population migration rates for *P. g. gaigeae* was significantly different from zero, and values are not presented in the figure. Time since divergence is represented on the *y*-axis, and the most probable time since divergence is illustrated in each figure. The estimated ancestral population sizes are represented by the width of the lower boxes, whereas the estimated current population size of the islet populations correspond to the width of the upper left box and those of the mainland populations to the upper right box. Estimated ancestral population sizes are also given below the population assignations. Grey arrows denote 95% posterior density intervals.

~500 m of sea water (Paulo *et al.* 2008). In the light of this, the finding of vicariance divergence over the small geographical scale in *Podarcis gaigeae* is surprising. The strong population structures between islets (Runemark *et al.* 2010; Fig. 2) and the pronounced morphological differentiation between mainland- and islet populations (Pafilis *et al.* 2009a) of *P. gaigeae* are consistent with vicariance though. This vicariance scenario with limited dispersal between populations is also consistent with the low effective population sizes and genetic drift acting (Runemark *et al.* 2010, 2011) as well as results from other *Podarcis* species in the Mediterranean (Castilla *et al.* 1998; Podnar *et al.* 2005; Paulo *et al.* 2008). This pattern is also consistent with patterns from some other taxa in the Aegean (see for example Sfenthourakis (1996) and Comes *et al.* (2008)) and supports vicariance divergence even over smaller geographical scales despite the evidence in favour of dispersal as the main force driving divergence (de Queiroz 2005; Cowie & Holland 2006).

As we have multiple comparisons and find a correlation between geologically and molecularly dated divergence times, we are able to independently estimate a microsatellite mutation rate and use this for calculating the demographic parameters. These analyses suggest an *c.* 5.8 times higher value for the mutation rate relative to our initial value of 0.0001. Both the mutation rate used in our runs (0.0001) and the mutation rate scaled by the factor from the regression between molecular and geological divergence time (0.00058) are within the expected mutation rates for microsatellites (Ellegren 2004). We have chosen to illustrate the effects of using different mutation rate scalars through calculating the demographic parameters for different mutation rate scalars (Tables S2–S4, Supporting information). In the text and figures, we present the demographic estimates based on the originally assumed mutation rate of 0.0001.

The estimated current population sizes were generally much larger on the mainland localities than on the islet localities (Table 2), which is expected because area limits population size in reptiles (King 1987). These results are also largely consistent in an earlier study (Runemark *et al.* 2010) where population size estimation based on linkage disequilibrium (Waples & Do 2008) was employed. The ancestral population sizes were estimated to be much larger than the current population sizes (Table 2), which is consistent with a larger landmass that subsequently became inundated, forming the Skyros archipelago.

Although the isolation of populations suggests the potential for vicariance speciation, we found significant nonzero estimates of gene flow from islets to mainland populations *P. g. weigandi* into the Palamari mainland population of *P. g. gaigeae*. This indicates that there is some dispersal in this vicariance scenario. The finding that there is a negative relationship between distance and gene flow from islets to mainland populations is also suggestive of gene flow. There is, however, no significant gene flow from *P. g. gaigeae* into *P. g. weigandi*,

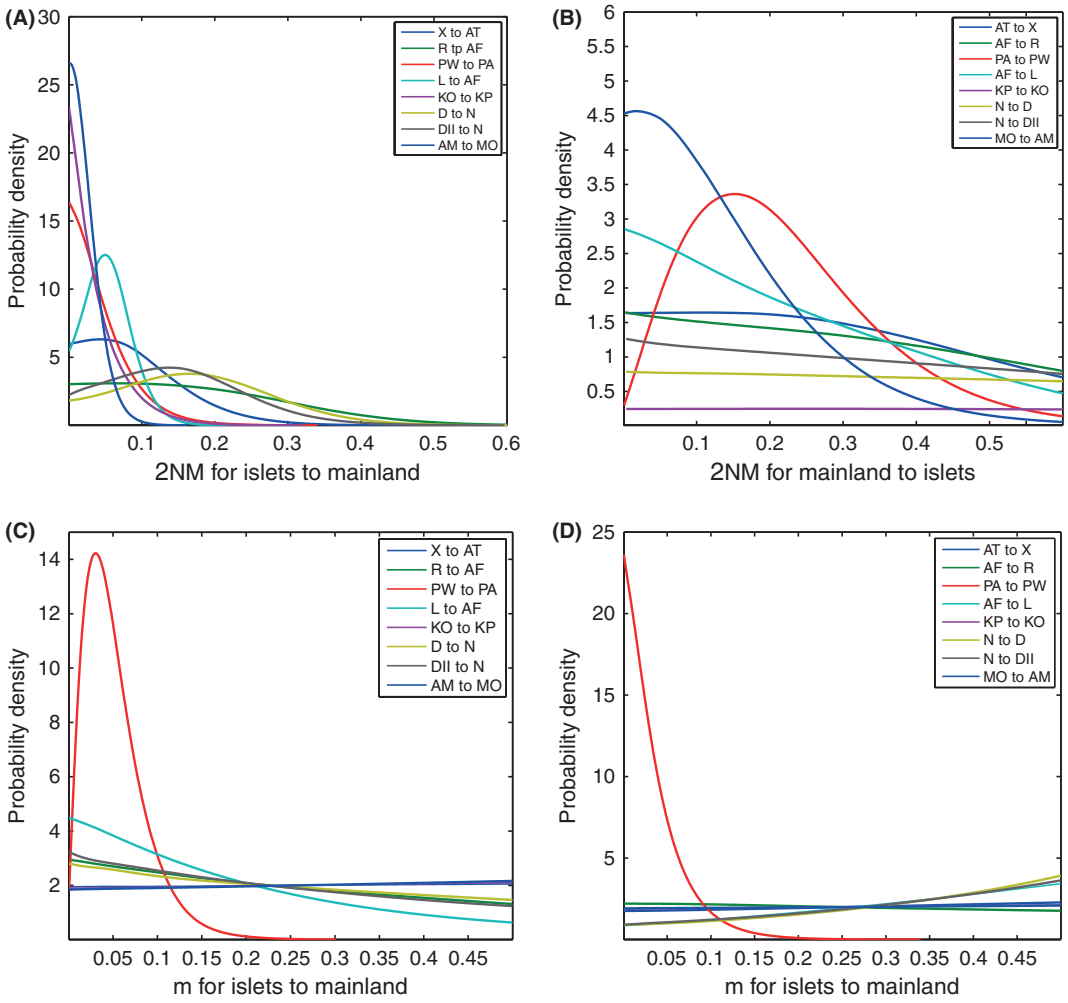

Fig. 5 Estimated marginal posterior densities for 2*NM* 'population migration rate' from islet to mainland (A) and mainland to islet (B), and estimated marginal posterior densities for *m*, migration rate per gene copy per generation, for islet individuals (C) and mainland individuals (D) respectively. Curves have been derived using a uniform prior. Results both between populations of *Podarcis g. gaigeae*, and between *P. g. gaigeae* and the subspecies *Podarcis g. weigandi* (depicted in red), are shown.

possibly due to different selective environments that favour migration in the former direction. Alternatively, migration in the former direction could be facilitated by, for example, sea current direction (cf. Calsbeek & Smith 2003). Although no consistent asymmetry in levels of gene was found, large differences were found in individual cases. For example, the gene-flow estimate was much less from the islet population KO to the mainland population KP than the reverse (Table 2; Fig. 1). Large asymmetries but with more gene flow from the mainland populations to the islets was found in the cases of DII-N and D-N though (Table 2; Fig. 1). Such asymmetries support the presence of gene flow in at least one direction.

We found indications of some gene flow between the islets and the mainland in an archipelago where the sea forms a very strong barrier to dispersal. Potentially, anthropogenic activities such as transportation of grazing goats (which occur on five of the seven islets) as well as church visits (one of the two nongrazed islets) could have contributed to this gene flow. *Podarcis* lizards have also been shown to cross sea barriers of similar magnitudes (Paulo *et al.* 2008). To avoid to construct an uninformative island model where divergence time and level of gene flow could not be resolved, we had to use a rather low migration prior for the islet populations, though (see discussion above). This implies that for some cases, the absolute population migration rate cannot be estimated. The very recent divergence of the islet populations might also imply that there has not been time for gene flow to shape divergence.

Although the exact amount of gene flow might not be easily measurable in the present study of recently diverged populations, the relative relationship between

gene-flow levels in the different directions can also be informative. We used a quantitative approach and found that population migration rate from islets to mainland was negatively correlated with distance between locals, and positively correlated with islet area across populations. These effects are probably due to that chances are higher to accidentally be flushed out and make it to the mainland from a large islet closer to the coast. We found no such relationship for the population migration rates from the mainland to the islets, though.

To conclude, our data suggest an ongoing vicariance divergence model in the Skyros wall lizard, where islet populations have sequentially become isolated because of sea level raise. We also find some evidence that gene flow has also acted to limit divergence, although recent gene flow has not been frequent enough to override the molecular signature of vicariance. Recent gene flow between the two subspecies suggests that even in this geographically allopatric scenario with an extremely inhospitable matrix (the sea), divergence with some dispersal and gene flow is still feasible. Our study reveals the potential for vicariance speciation and genetic drift as strong forces promoting population divergence in the Aegean Archipelago, as has been suggested for some other taxa (Sfenthourakis 1996; Comes *et al*. 2008).

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Data accessibility

Sample locations and microsatellite data are deposited at DRYAD entry doi:10.5061/dryad.3b5q2fm6.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Pair-wise F_{ST} for all studied populations.

Table S2 Molecularly estimated divergence times (years) between islet- and mainland populations of the Skyros wall lizard for different assumed mutation rates.

Table S3 Estimated population sizes (number of individuals) for islet- and mainland populations of the Skyros wall lizard for different assumed mutation rates.

Table S4 Estimated migration rates (2*NM*) between mainland- and islet population and between islet- and mainland populations of the Skyros wall lizard respectively, for different assumed mutation rates.

Table S5 Estimated divergence times (years) under a mutation rate of $\mu = 0.0001$ and the 95% low- and high credibility intervals respectively.

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