LETTER

Sexual selection drives asymmetric introgression in wall lizards

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Abstract

Hybridisation is increasingly recognised as an important cause of diversification and adaptation. Here, we show how divergence in male secondary sexual characters between two lineages of the common wall lizard (*Podarcis muralis*) gives rise to strong asymmetries in male competitive ability and mating success, resulting in asymmetric hybridisation upon secondary contact. Combined with no negative effects of hybridisation on survival or reproductive characters in F1-hybrids, these results suggest that introgression should be asymmetric, resulting in the displacement of sexual characters of the sub-dominant lineage. This prediction was confirmed in two types of secondary contact, across a natural contact zone and in two introduced populations. Our study illustrates how divergence in sexually selected traits via male competition can determine the direction and extent of introgression, contributing to geographic patterns of genetic and phenotypic diversity.

INTRODUCTION

Gene transfer between species, sub-species or genetic lineages via hybridisation is increasingly recognised as an important cause of diversification and adaptation (Arnold 2007; Abbott *et al*. 2013; Hedrick 2013). Because hybridisation does not necessarily lead to an even mix of genetic and phenotypic characters of the parental lineages, it can cause new characters to arise or existing characters to be unequally transferred between lineages. This may be particularly likely when phenotypes that have diverged in allopatry confer a fitness advantage to one lineage upon secondary contact, making it advantageous for the other lineage to express the same characters. For example, expression of hetero-specific characters can have a survival advantage, which has been suggested to explain introgression of wing patterns between *Heliconius* butterflies (Pardo-Diaz *et al*. 2012), pest resistance in mice (Song *et al*. 2011) and the evolution of climate adaptation and herbivore resistance in sunflowers (Whitney *et al*. 2006, 2010). Alternatively, characters that confer a reproductive advantage in the competition for mates can enhance hybridisation rates as well as provide hybrids with a selective advantage relative to subdominant pure-bred competitors. In the absence of severe genetic incompatibilities, this may enable secondary sexual characters to rapidly spread from one lineage to another (Parsons *et al*. 1993; Prado *et al*. 2009; Baldassarre *et al*. 2014).

Sexually selected hybridisation has primarily been studied with respect to female choice. While female choice will often restrict gene flow (e.g. Saetre *et al*. 1997; Seehausen *et al*. 2008), increasing evidence suggests that it can also lead to asymmetric rates of hybridisation and introgression of male sexual characters (Wirtz 1999; Stein & Uy 2006; Pfennig 2007). For example, in a hybrid zone between the golden-collared (*Manacus vitellinus*) and white-collared (*Manacus candei*) manakins, females prefer golden-collared males on mixed leks, which results in asymmetric introgression of golden plumage colouration across the hybrid zone (Parsons *et al*. 1993; Stein & Uy 2006). In contrast, evidence that divergence in sexual characters conferring an advantage in male–male competition can promote asymmetric gene flow between lineages is very limited (Hedrick 2013). This is despite that competition between males for resources is important for the evolution of character displacement and reproductive isolation (Grether *et al*. 2013), and hence features frequently in speciation theory (Price 2008). Behavioural experiments suggest that competitive exclusion of males of the sub-dominant lineage may contribute to the golden-collared male mating advantage at mixed leks in manakins (McDonald *et al*. 2001), cause differences in the frequency of hetero-specific pairings between pied and

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collared flycatchers (Vallin *et al.* 2012) and between hermit and Townsend's warblers (Pearson 2000). However, even in these relatively well-studied systems the link between intraspecific sexual selection and genetic and phenotypic introgression remains largely circumstantial.

We studied how behavioural interactions as well as post-copulatory and post-zygotic reproductive isolation influence gene flow between two phenotypically distinct lineages of the common wall lizard, *Podarcis muralis*. This species has formed a number of genetic lineages in Southern Europe reflecting isolation in ice age refugia (Salvi *et al.* 2013). Our focus was on lizards native to Western Europe, which correspond morphologically to the *P. muralis brongniardii* subspecies, and on lizards native to northern Italy (Tuscany), which correspond morphologically to the *P. muralis nigriventris* subspecies (Böhme 1986). These lineages now form a natural contact zone in Liguria (northwestern Italy, see Results) and have also come into secondary contact more recently as a result of human introductions in both Germany and southern England (Schulte *et al.* 2012a; Michaelides *et al.* 2013).

We used an experimental approach to generate predictions regarding how natural and sexual selection should influence the direction of introgression, followed by genetic and phenotypic analyses in all three regions of secondary contact to test these predictions. First, we conducted an extensive phenotyping of lizards from the two main lineages in allopatric native populations to establish the extent to which they exhibit divergence in sexually selected characters. Second, we used experimental populations in outdoor enclosures to test whether such differences translate into an asymmetry in male dominance and realised hybridisation upon secondary contact. Third, we assessed the reproductive compatibility of between lineage crosses and the survival and reproductive competence of F1 hybrids. Finally, we made use of these data to predict the direction of introgression, which we tested using mitochondrial DNA, microsatellites and phenotypic data across three locations (one native and two introduced) of hybridisation.

METHODS

Common wall lizards are small, 45–75 mm snout-to-vent length, diurnal lizards that inhabit a range of natural and anthropogenic habitats. We studied native populations in France, western Germany, and northwestern Italy, which collectively belong to a mitochondrial lineage that we here refer to as the western European lineage, and populations in northern Italy (Tuscany), which we refer to as the Italian lineage (Schulte *et al.* 2012a). The data in this paper also involve 11 introduced populations in England of single native origin, and two introduced populations of mixed origin (i.e. presence of animals of both western European and Italian origin or hybrids), one in England and one in Germany. Further details on the populations are found in Table S1.

Character divergence in allopatry

We collected morphological and colouration data on 793 animals from 31 native populations of pure Western European and Italian origin. We captured all lizards by noosing,

weighed them to the nearest 0.01 g and measured their snout-to-vent length, total length, head length and head width to the nearest mm. Using photographs, animals were scored for ventral (blackness) and dorsal (greenness) colouration. Ventral blackness was scored by quantifying the proportion of black to non-black pixels on each lizard's chest (Fig. S1). Dorsal greenness was scored based on an intensity scale from 1 to 10 (1 being *pure brown*, 10 being *pure green*, Fig. S2), which was confirmed to be highly correlated with scores from digital photographs analysed in Photoshop CS4 and with values for green chroma extracted using spectrophotometry [see Supporting Information (SI) for full details]. We also collected data on bite force and male testes mass, which are both commonly under sexual selection in lizards (Olsson & Madsen 1998; see SI for full details and sample sizes).

Patterns of dominance, courtship and paternity upon secondary contact

To generate predictions regarding the direction of hybridisation, we carried out two separate experiments using outdoor enclosures (7 × 7 m), designed to simulate conditions during secondary contact. Each enclosure was fitted with suitable habitat (bricks, wooden pallets) and stocked with 16 animals, four males and four females of each lineage (with the exception of four enclosures in 2010; see SI for details). In 2010, 10 enclosures were stocked with a total of 160 animals of either Italian or Western European origin sourced from introduced populations in England. In 2013, we conducted a similar experiment using eight enclosures stocked with a total of 128 animals captured from native populations in western France or Tuscany. In both experiments, we collected individuals from multiple populations (*n* = 10 in 2010 and *n* = 7 in 2013). To reduce population-of-origin effects, animals from the same source population were distributed among all enclosures as evenly as possible. Within this constraint the location of each individual was assigned randomly. The two experiments differed slightly in the distribution of habitat within enclosures, but followed the same protocol for data collection (see SI for full details).

Individuals were captured from the wild prior to females laying their first clutch and were transported to the laboratory. The experiments were conducted following oviposition of the first clutch (females typically lay at least two clutches per season). All individuals of each sex were released into a given enclosure at the same time. Males were released first to allow them to establish territories, followed by females (~ 7 days between the release of males and females). Females released more than 3 days after oviposition were kept cool (~ 10 °C) during this period to avoid progression through the next ovulation cycle. Behavioural interaction data were obtained throughout the experiment from rotating 45 min observation periods per enclosure, conducted by three (in 2010) or two (in 2013) observers in an ethogram (Table S2, see SI for full details). This resulted in a total observation period of ~ 510 h in 2010 and ~370 h in 2013.

Once females were ready to lay, all individuals were recaptured and returned to cages in the laboratory. Cages were inspected in the morning and late afternoon for signs of egg

laying. Eggs were incubated at a constant 24 °C (2010) or 28 °C (2013) in standard refrigerated incubators fitted with water baths to maintain humidity. At hatching, offspring were euthanised (using concussion followed by permanent destruction of the brain) and their tissues used for genetic analysis. DNA was isolated from tail–tip tissue using standard protocols (see SI for full details). Paternity was assigned using microsatellites (Table S3) in CERVUS v 3.0 (Marshall *et al.* 1998) based on the trio (mother, father, and offspring) LOD score and a strict confidence level of 95%.

To confirm whether these patterns were the result of pre- as opposed to post-copulatory mechanisms, we carried out 16 sperm competition trials in the laboratory in which Western European ($n = 6$) and Italian ($n = 10$) females were mated to males of both their own and the other lineage. All trials were carried out in the same type of terrarium used for housing the animals (see above) in the first 5 days following oviposition, which corresponds to the female receptive period under laboratory conditions. Females were introduced to the terrarium and allowed to acclimatise for 20 min after which one of the males was introduced to the female. Once mated (all within an hour) that male was removed and the second male was immediately introduced (all also mated within an hour). The order of males with respect to lineage was reversed each trial. Offspring were genotyped along with their mother and the two potential fathers as described above.

Fertility and viability of F1 hybrids

To test for decreased hybrid fitness we carried out 62 crosses between males and females of the two lineages. We introduced a male of either the same lineage or the other lineage into a female cage 3 days after she had laid her first clutch and left them together for 5 days. Eggs were collected following oviposition, scored for infertility based on presence and calcification of the egg shell (Olsson & Shine 1997) and incubated at 24 °C. Embryonic mortality was scored and assessed using dissection of eggs that did not show any evidence of heart beat (using a digital egg monitor: Buddy; Avitronics, Truro, England). Ninety-six offspring from these crosses were raised to maturity under laboratory conditions. After reaching mature size (~ 5 months after hatching) they were hibernated for 10 weeks at 4 °C. For logistical reasons we were unable to conduct further crosses to establish a F2 generation and therefore assessed reproductive capacity under captive conditions for a subset of animals. We recorded whether or not females produced eggs within 2 months of emergence of hibernation and the resulting clutch size. We also recorded the testes mass of 23 males. These characters should reflect fertility of F1 hybrids, but it should be noted that it does not establish sperm characteristics in males and that incompatibilities may not be evident until the F2 generation; our data may therefore underestimate genetic incompatibilities in hybrids.

Statistical analyses

All data were analysed using R version 3.0.3 (R Development Core Team 2010). We used linear (mixed) models to analyse differences between lineages and sexes in phenotypic charac-

ters in both wild-caught animals and experimental crosses and to establish patterns of behaviour and parentage in the experimental enclosures. Detailed description of all models can be found in the SI.

Genetic and phenotypic patterns in regions of secondary contact

We examined phenotypic and genetic patterns of introgression within three separate regions of secondary contact between the Italian and Western European lineage. In the native hybrid zone, we sampled 17 populations from central Tuscany (where animals are known to fall within the Tuscan haplotype lineage; *sensu* Schulte *et al.* 2012a; and exhibit *P. m. nigriventris* phenotypes) to western Liguria (where animals are known to belong to the western European haplotype lineage and exhibit typical *P. m. brongniardi* phenotype) (Böhme 1986) (Fig. 1). Second, we sampled 27 animals from a location in England (Holmsley) that is known to have both Italian and Western European origins (Michaelides *et al.* 2013). Third, we sampled 203 animals from a population in south-western Germany (Mannheim) where animals from the Italian lineage have been introduced in a region where the western European lineage is native (Schulte *et al.* 2012b). In each of these populations, we recorded traits as described above and removed ~ 5 mm of the tail or took buccal swabs for DNA analyses.

In the native hybrid zone, we tested predictions regarding the direction of gene flow using a geographic cline approach (Szymura & Barton 1986; Gay *et al.* 2008). Because microsatellite loci were highly variable and showed few private alleles, typically at low frequencies, we estimated the nuclear genetic cline from a Bayesian hybrid index (HI) based on allele frequencies at all loci using the programme STRUCTURE v 2.3.4 (Pritchard *et al.* 2000). Because phylogeographic studies have established two lineages in this geographic region we conducted all analyses assuming two genetic clusters (i.e. $K = 2$). The simulations, using the admixture model, run with a burn-in of 10^5 iterations and a further run length of 10^6 iterations. Runs were replicated five times and combined using CLUMPP (Jakobsson & Rosenberg 2007). We used the probability that an individual was assigned to the Italian cluster (Q) as our hybrid index. The hybrid index was subsequently used to assign individuals as either pure Western European ($Q \leq 0.1$), pure Italian ($Q \geq 0.9$) or hybrid ($0.1 < Q < 0.9$) (e.g. Baldassarre *et al.* 2014). We also fitted the corresponding cline for haplotypes based on the cytochrome *b* mitochondrial gene. Phenotypic clines using population averages were fitted for three traits: dorsal greenness, ventral blackness and relative head length. These are all quantitative characters with large and well-established differences between lineages (greater in the Italian lineages, e.g. Böhme 1986, Fig. S3; see Results). Relative head length was calculated as the residual score from a regression of head length on snout-to-vent length. Clines were treated separately for males and females.

Genetic and phenotypic clines were fitted using the Metropolis-Hastings Markov chain Monte Carlo algorithm implemented in the package hzar in R version 3.0.3 (Derryberry *et al.* 2014). For the genetic analyses we ran two sets of five models. Each model estimated cline centre (cumulative distance from sampling location Colle di Val D'Elsa in Tuscany, *c*) and width (1/maximum slope, *w*), but could also fit
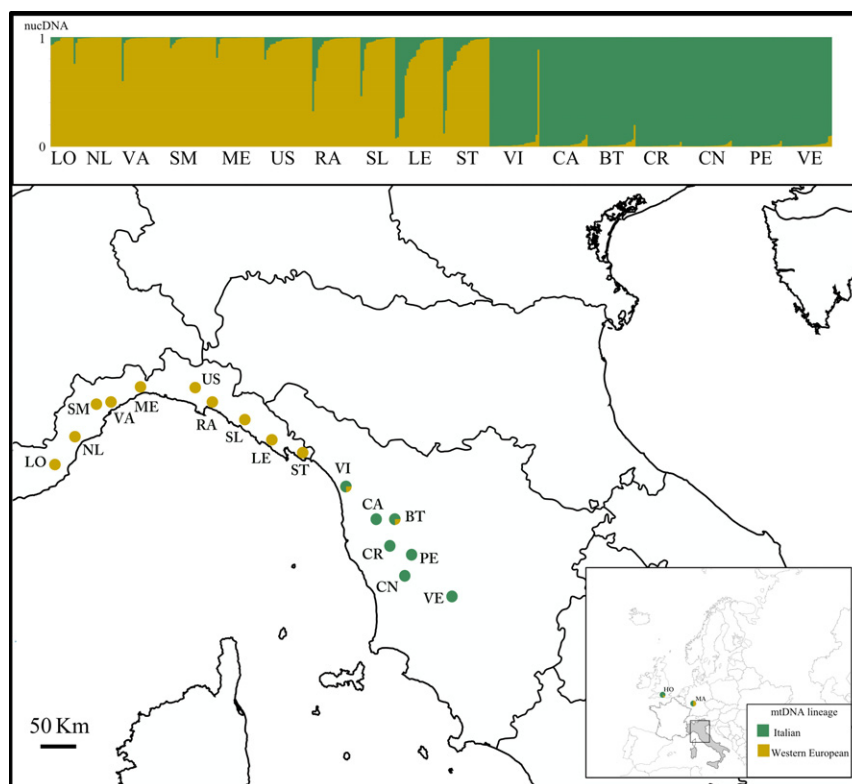
Figure 1 Map of the native hybrid zone in northern Italy. We used 17 populations that created a cline from mid Tuscany up the Ligurian coast. The colour of the dots indicates the association with a particular mitochondrial lineage. Two populations in the middle of the cline contained a mix of haplotypes (VI and BT). At the top of the figure a STRUCTURE output indicates the extent of admixture (using microsatellite nuclear DNA) within these populations as a function of distance from the far western end of the cline (running left to right). Insert shows the location of the geographic region and the two hybrid regions in England (Holmsley) and Germany (Mannheim).

different combinations of the exponential decay curve parameters δ and τ (none, right tail only, left tail only, mirrored tails, or both tails separately). One set of models fixed the cline ends at the empirically observed values, whereas the other set also estimated these values from the data. We also ran the corresponding models for each of the phenotypic traits, but because of small sample size (< 10) for the Westernmost population, the models with fixed cline ends used the value of the closest population (i.e. Noli, NL). For each of the clines we compared models based on the AIC corrected for small sample size (AICc) and selected the model with the lowest AICc as the best-fitting model. The coincidence of cline centres for mtDNA vs. ncDNA, and for ncDNA vs. phenotypic clines was assessed using the maximum-likelihood derived confidence intervals, where we considered non-overlapping confidence intervals as statistically supported differences in cline location. We verified the conclusions by re-fitting models that constrained the cline centre to correspond to that of the cline to which it was compared.

For the two non-native populations we tested for the presence of hybrids and the direction of hybridisation. First, we conducted a principle coordinate analysis (PCoA) to visualise pair-wise individual multi-locus genetic distance calculated in GenAlEx (Peakall & Smouse 2012). For the non-native population in England, we included four non-native populations of pure origin (three of Italian and one of Western European

origin) that served as source populations (Michaelides *et al.* 2015). For the non-native population in Germany (Mannheim) we did not include reference populations as the exact origins are unknown. Instead, we pooled individuals into two groups based on the lineage assignment from the cytochrome *b* gene (Western European and Italian). Second, we conducted Bayesian assignment tests to identify individuals of mixed origin. We used the admixture model as implemented in STRUCTURE to assign individuals as either pure Western European ($Q \leq 0.1$), pure Italian ($Q \geq 0.9$) or hybrid ($0.1 < Q < 0.9$). We also did the corresponding analysis in the programme NewHybrids (Anderson & Thompson 2002), which computes posterior probabilities of individual assignment into different categories of hybrids based on their multi-locus genotypes (F1 or F2). We used uniform priors with 10^5 sweeps before and 10^6 sweeps after burn-in. The direction of hybridisation was assessed by comparing the outcome of these assignment tests to the mitochondrial haplotype.

RESULTS

Character divergence in allopatry

Lizards from the Italian lineage displayed the characteristic green dorsal and black ventral colouration typically ascribed to *P. m. nigriventris* and had larger heads, stronger bite

force and greater testes mass (Table S4; Fig. 2). Sexual dimorphism was generally greater in the Italian lineage (Table S4, Fig. 2).

Patterns of paternity upon secondary contact

We found highly consistent results across both experiments. Italian males were strongly dominant over Western European males, winning more agonistic interactions (permutation test using QAP – 2010, lineage: $P = 0.019$, snout-to-vent length: $P = 0.012$, QAP – 2013, lineage: $P < 0.001$, snout-to-vent length: $P = 0.05$; Fig. 3a). Across both experiments, dorsal greenness, ventral blackness and head length, phenotypic characters that are exaggerated in Italian males, were all strong phenotypic predictors of dominance (Table S5).

Italian males courted significantly more females (Table S6; Fig. 3b), and had higher reproductive success overall and with females of the opposite lineage, than Western European males (Table S6; Fig. 3c). Accordingly, Western European females produced a significantly higher proportion of hybrid offspring compared to Italian females (Table S6; Fig. 3d). Rerunning models including dominance as a predictor suggested that differences in reproductive success between Italian and Western European males were well explained by dominance and hence consistent with male–male competition (Table S7).

Males were more likely to sire offspring with females from their own lineage under sperm competition (intercept: $\chi^2 = 8.45$, d.f. $= 1$, $P < 0.01$), but there was no statistical support for a bias in reproductive success with females from the other lineage between Western European and Italian males (proportion of hybrids in Italian clutches $= 0.21 \pm 012$, proportion of hybrids in Western European clutches $= 0.42 \pm 0.20$, lineage: $\chi^2 = 0.22$, d.f. $= 1$, $P = 0.64$).

Fertility and viability of F1 hybrids

Embryonic mortality was not higher for between-lineage crosses (17%) compared to within-lineage crosses (16%) (male lineage: $\chi^2 = 3.19$, $P = 0.07$, female lineage: $\chi^2 = 3.19$, $P = 0.43$, male lineage × female lineage: $\chi^2 = 0.42$, $P = 0.51$). Animals of pure Western European origin had slower growth rates and were smaller following hibernation than animals of Italian and hybrid origin (Western European offspring $= 47.3 \pm 1.19$ mm, Italian offspring $= 52.5 \pm 0.78$ mm, Hybrid offspring $= 51.6 \pm 0.64$ mm, cross: $\chi^2 = 245.3$, $P < 0.001$, sex: $\chi^2 = 16.8$, $P = 0.32$). We found no significant difference between the crosses in testes mass for a given body size (Western European males $= 0.017 \pm 0.01$ g, Italian males $= 0.035 \pm 0.01$ g, Hybrid males $= 0.031 \pm 0.01$, cross: $\chi^2 = 1.81$, $P = 0.24$, snout-to-vent length: $\chi^2 = 0.07$, $P = 0.72$). Captive-reared females of French origin did not reproduce, but female hybrids were as likely to reproduce as pure-bred Italian females (36% of Italian females reproduced vs. 45% of hybrid females: $\chi^2 = 0.03$, $P = 0.85$), and there was no significant difference in clutch size (Italian females $= 3.20 \pm 0.58$, Hybrid females $= 3.55 \pm 0.17$: $\chi^2 = 0.51$, $P = 0.48$).

Genetic and phenotypic patterns in regions of secondary contact

The results above predict that introgression should be male-driven and asymmetric from the Italian lineage into the
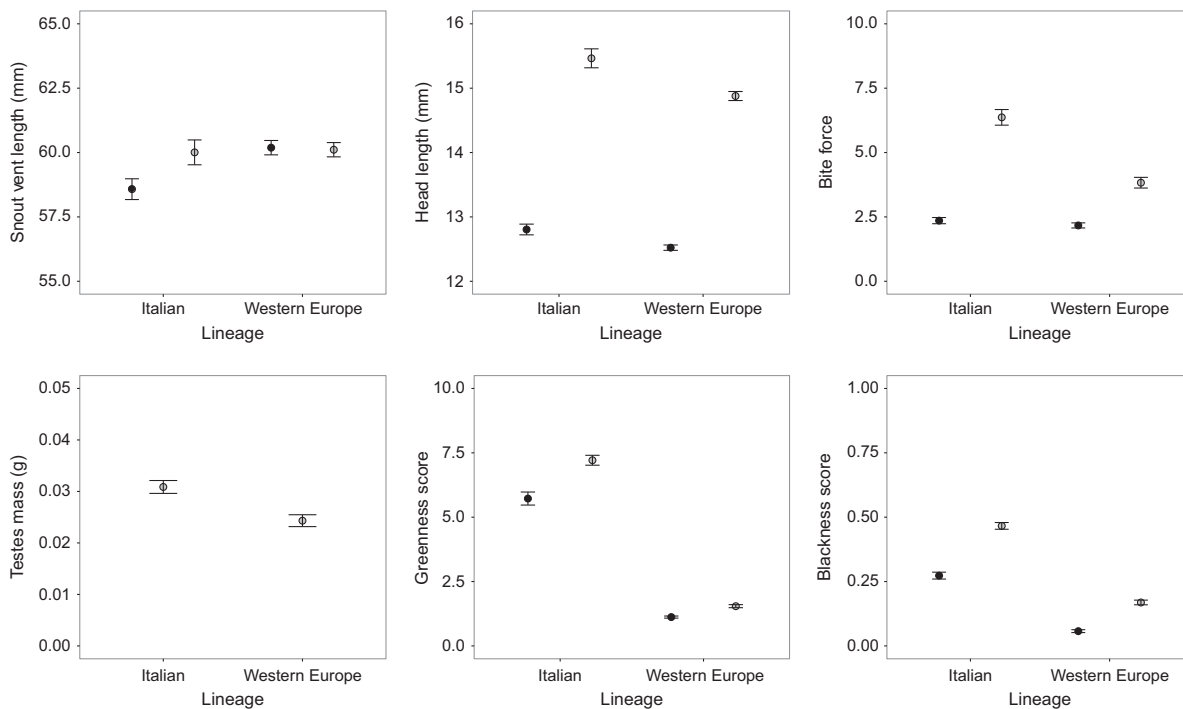

Figure 2 Means ± SE for Western European and Italian animals in morphological and colour phenotypes. Black dots indicate females and grey dots indicate males.
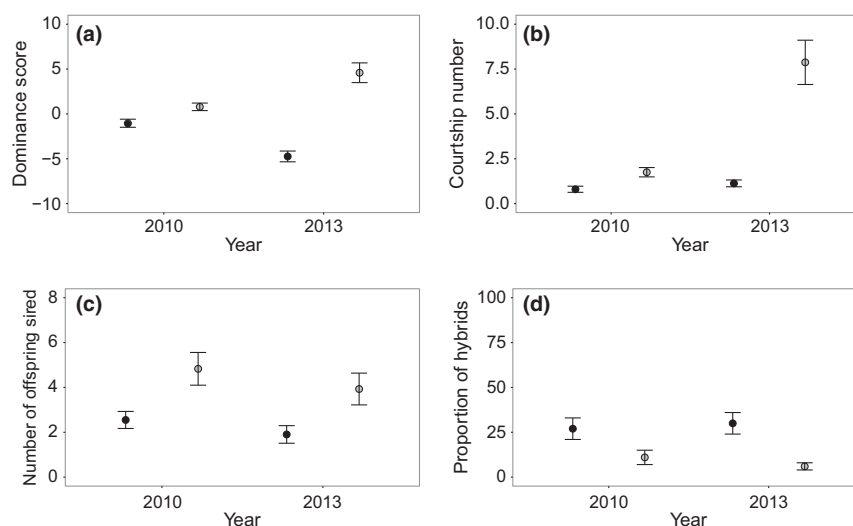
Figure 3 Means ± SE for Western European and Italian lizards in male dominance, number of courtships males initiated, the number of offspring males sired and the proportion of a females clutch that were hybrids. All outputs are the result of our experimental secondary contact zone experiments carried out with non-native (2010) and native (2013) lizards. Black dots indicate Western European lizards and grey dots indicate Italian lizards. Note that the higher reproductive success of Italian males (panel c) is the result both of a greater number of clutches with paternity assignment for Italian females and higher reproductive success with females of the opposite lineage (panel d).

Western European lineage. As predicted, the location of the geographic cline for microsatellites was shifted westwards compared to the cline for mtDNA (Table 1; Fig. 4). A microsatellite cline with its centre constrained to that of the mtDNA cline provided a significantly worse fit to the data (ΔAICc = 43.1). The locations of both genetic clines were significantly different from the cline centres for all three phenotypic traits (dorsal greenness Fig. 4, ventral blackness and relative head length Fig. S4), which were shifted even further to the west (Table 1). Notably, we failed to identify significant levels of genetic admixture for several of the western-most populations with Western European haplotypes (e.g. US, RA, SL; Fig. 1) that were phenotypically very similar to populations identified as being of pure Italian origin (Fig. 4). The best-fitting models for the phenotypic clines differed between traits and, for black ventral colouration, between the sexes (Table S8). There was a significant correlation between genetic

differentiation and geographic distance ($r^2 = 0.70$, $P < 0.001$, Fig. S5).

STRUCTURE assigned all individuals in the putative hybrid population in southern England as being of pure Italian origin despite four individuals having mtDNA haplotypes from Western Europe (Fig. S6). The results were corroborated by the output from NewHybrids in which all individuals were assigned as being pure Italian (Table S9) and the PCoA in which hybrid individuals were found within the cluster of pure Italian individuals (Fig. S7). In Mannheim, STRUCTURE identified 23 out of 203 individuals as hybrids ($0.1 < Q < 0.9$; Fig. S8) and NewHybrids tended to classify these as being F2 hybrids (e.g. F1 × F1 hybrids; Table S11). Eight hybrid individuals in Mannheim harboured Italian mtDNA haplotypes and the rest (15) had mtDNA haplotypes from Western Europe (Table S10). The PCoA placed these within and/or between the clusters of pure individuals (Fig. S9).

Table 1 Parameter estimates for best-fitting cline models for genetic and phenotypic clines using *HZAR* (Derryberry *et al.* 2014). Parameter *c* indicates the estimated cline centre (distance from sampling location VE in Tuscany) and *w* indicates the cline width (1/maximum slope). The parameters *pmin* and *pmax* indicate the allele frequencies at the ends of the cline for genetic markers and the corresponding values for phenotypic markers (transformed values to the second decimal point), and δ and τ are exponential decay curve parameters for the left and right tails. Two log-likelihood unit support limits are presented in parentheses. Note that the very high introgression of phenotypic characters makes the parameter estimates for the fit in the western part of the cline unreliable (see Fig. 4; Fig. S4)

| Character	Sex	Best model	*c* (km)	*w* (km)	*pmin*	*pmax*	δL	τL	δR	τR
mtDNA		Model I	61.2 (56.0–68.8)	29.9 (18.3–50.3)	0	1	None	None	None	None
Hybrid Index		Model VII	100.5 (88.7–118.7)	15.2 (1.2–50.2)	0	1	None	None	2.37 (0.0–18.2)	0.131 (0.011–0.705)
Greenness	M	Model II	273.0 (254.3–278.0)	76.1 (56.2–93.6)	1.44	2.71	None	None	None	None
Greenness	F	Model II	228 (225.7–230.0)	5.1 (3.7–5.9)	0.07	1.82	None	None	None	None
Blackness	M	Model II	156.2 (124.3–279.8)	105.7 (20.1–309.3)	0.18	0.45	None	None	None	None
Blackness	F	Model VIII	226.6 (219.7–231.5)	22.1 (14.7–56.7)	0.06	0.23	None	None	292.4 (1.2–307.4)	0.743 (0.006–0.972)
Head length	M	Model I	213.2 (207.2–222.8)	14.8 (0.1–30.2)	−0.38	0.14	None	None	None	None
Head length	F	Model I	227.5 (215.2–238.9)	62.6 (25.4–125.7)	−0.72	0.08	None	None	None	None
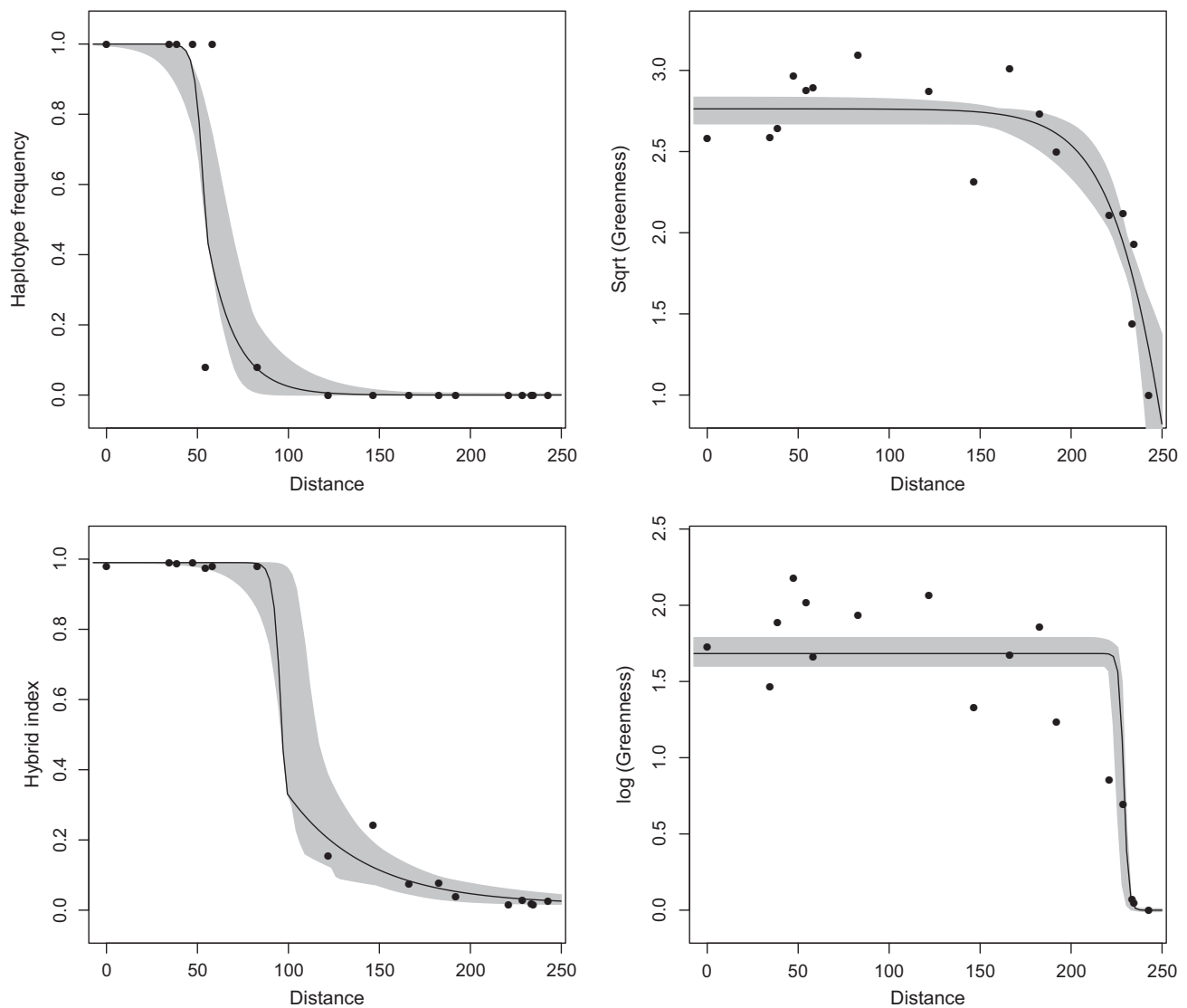
Figure 4 The maximum-likelihood cline and the 95% credible cline region for best-fitting models (Table 4) for mtDNA haplotype (top left), hybrid index (bottom left) and the lineage-characteristic dorsal colouration for males (top right) and females (bottom right). Greenness of dorsal colouration was scored on a scale of 1–10 and was transformed to improve fit to model assumptions (square root and logarithmic transformation for males and females respectively). Transect distance is the cumulative distance from the south-easternmost population Colle di Val D'Elsa in Tuscany with increasing distance westwards.

DISCUSSION

The evolutionary consequences of secondary contact should depend on the genetic and phenotypic divergence between lineages. Our results show that divergence in male competitive ability in allopatry causes asymmetric hybridisation and gene flow upon secondary contact in wall lizards. As a consequence, sexually selected introgression shapes phenotypic and genetic variation in both native and non-native populations.

Wall lizards in north-central Italy show exaggeration of characters that are under sexual selection in this (Sacchi *et al.* 2009) and other lizard species (Olsson & Madsen 1998). Our experiments show this is associated with an advantage in male–male competition for females, leading to an overall

greater courtship, mating success and increased rates of hybridisation with females of the other lineage compared to males from the Western European lineage. These patterns are unlikely to be mediated by female choice as we have shown elsewhere that females do not discriminate between males of different lineages, whereas males prefer females from their own lineage (Heathcote 2013). Post-copulatory mechanisms also appear unlikely to explain patterns of paternity as there was no evidence for a competitive advantage for Italian males in sperm competition trials. Our results therefore suggest that male–male competition and male mate choice should drive patterns of genetic exchange in zones of secondary contact. This is in contrast with the majority of previously studied vertebrates, where female choice is believed to be both the primary barrier to hybridisation as well as the major cause of

asymmetric introgression of male characters (Wirtz 1999; Stein & Uy 2006; Pfennig 2007).

Even if small differences in viability or fertility remained undetected in our experimental crosses, the differences in male competitive ability should create asymmetric introgression; that is male-driven gene flow from the Italian lineage into the Western European lineage. Data from the native hybrid zone provide strong support for this prediction. Nuclear microsatellite markers revealed a westward shift in the position of the hybrid cline compared to mitochondrial markers. As a result virtually all hybrids exhibited Western European haplotypes. Furthermore, the phenotypic clines were shifted even further westwards, such that several populations that were genetically (i.e. based on both ncDNA and mtDNA) assigned to the Western European lineage were phenotypically indistinguishable from pure populations of the Italian lineage. Even if head size and dorsal and ventral colouration are not the direct targets of sexual selection, our enclosure experiments show that these characters are strong predictors of male dominance, a robust predictor of reproductive success. Thus, the stronger introgression of phenotypic characters compared to microsatellite markers imply that these characters not only bias the direction of hybridisation, but are also selectively favoured within the hybrid zone. However, analysis of selection at the leading front of the hybrid zone would be necessary to establish ongoing selection on male secondary sexual characters.

The results from the native hybrid zone were supported by genetic data from two locations where at least one of the lineages has been introduced. These patterns were weaker than those observed in the native zone, potentially because of strong founder effects that are likely to have occurred during establishment. Nevertheless, in both of the non-native populations the mitochondrial–nuclear discordance was consistent with hybridisation being primarily between males of the Italian lineage and females of the Western European lineage. Thus, the results from all three regions of secondary contact point towards asymmetric introgression and displacement of male characters of the less dominant lineage by intrasexual selection (Schulte *et al.* 2012b), providing evidence that introgression can be a source of secondary sexual characters.

Is male–male competition a general mechanism of directional introgression? Differences in male competitive ability are commonly invoked to explain displacement of one species by another in sympatry (Grether *et al.* 2013). This could promote asymmetric hybridisation by making males of one species rare relative to females (Hubbs 1955). This mechanism is supported by studies of interspecific competition over nest sites in flycatchers (Vallin *et al.* 2012), but introgression in this species is very limited due to low hybrid fitness (Veen *et al.* 2001). Species-specific male aggression is also consistent with the direction of introgression of plumage colour in manakins (McDonald *et al.* 2001), the movement of hybrid zones between hermit and Townsend's warblers (Peason & Rohwer 2000) and between two species' of house mice (Teeter *et al.* 2007). Nevertheless, the best evidence that sexual selection drives introgression still comes from studies of female choice (Parsons *et al.* 1993; Stein & Uy 2006; Baldassarre & Webster 2013; Baldassarre *et al.* 2014). This could partly be because

of taxonomic bias. In lizards, male–male competition appears to be a stronger driver of variation in male reproductive success than female choice (Olsson & Madsen 1995). We therefore suggest that male–male competition often will be more important for the strength and direction of gene flow in lizard hybrid zones compared to, for example, bird hybrid zones.

The clines we observe in wall lizards are wider relative to the species dispersal ability than in other studies of sexually selected introgression (e.g. *Manacus* sp.; Uy & Stein 2007). In manakins, plumage introgression has been suggested to be limited by either habitat, which influences the conspicuousness of colour and geographically limits the benefit of golden plumage (Uy & Stein 2007), or by geographic barriers to dispersal (McDonald *et al.* 2001). In contrast, the habitat across the hybrid zone in the wall lizards typically consists of rocks and manmade structures (e.g. dry-stone walls) and geographic differences in the properties of this habitat are unlikely. Thus, there may be no limit to introgression along the coast in northwestern Italy and the geographic cline may be best viewed as a snapshot of an ongoing process of adaptive introgression that will eventually replace the phenotypes of the Western European lineage in this part of the species' distribution. In the introduced populations, we expect the formation of a hybrid swarm biased towards Italian characteristics, a process that evidently has already taken place in Holmsley.

Despite the close fit between our experimental data and the genetic and phenotypic clines, discordances between markers could also arise for a number of other reasons. We can refute most, if not all, of these for the native hybrid zone. First, the geographic scale of the discrepancy, compared to species dispersal distances, makes sex differences in dispersal highly unlikely as the cause of asymmetric introgression (Petit & Excoffier 2009). Lizard densities are also uniformly high across the zone. Second, we found no evidence that hybrid females are sterile, which rules out loss of fitness in female hybrids explaining differences in the mitochondrial and nuclear genetic clines (as expected from Haldane's rule; Haldane 1922). Third, environmental differences cannot explain the geographic pattern of phenotypic variation since the lineage differences persist in non-native populations and in captivity. Finally, the quantitative nature of the phenotypic characters means that it is unlikely that we are observing stochastic variation in introgression of loci across the genome, as could be the case for characters controlled by a single locus (e.g. colour polymorphisms; Mundy 2005).

In summary, we provide strong evidence that divergence in sexually selected traits in allopatry drives asymmetric hybridisation in wall lizards. This creates pronounced discordance between the phylogeography inferred from genetic markers and geographic patterns of phenotypic variation across multiple zones of secondary contact. These results suggest that, where post-reproductive isolation evolves slowly and female choice on male quantitative traits is absent or weak (as in lizards; Olsson & Madsen 1995), male–male competition may be an important cause of asymmetric introgression. This can lead to rapid introgression of potentially advantageous alleles and traits between species and ultimately promote novel genetic and phenotypic diversity in recipient populations.

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AUTHOR CONTRIBUTIONS

TU and GMW conceived of, designed, and organised the research. TU, GMW, SM, HEAM, NZ, PC, GPL, RS and MALZ conducted field work in Italy, France and England. JB conducted field work in Germany. RJPH and HEAM carried out the enclosure experiments, including assignment of parentage. TU, GMW, HEAM, TH and BF collected data from other experiments on captive animals. SM, NZ and HEAM generated the mtDNA and microsatellite data from native and UK populations. JB did the corresponding part for animals from the German populations designed and supervised by US, MV and AH. TU, GMW, RJPH, SM and JB analysed the phenotypic and genotypic data. TU and GMW wrote the paper with input from all authors.

REFERENCES

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N. *et al.* (2013). Hybridization and speciation. *J. Evol. Biol.*, 26, 229–246.

Anderson, E.C. & Thompson, E.A. (2002). A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, 160, 1217–1229.

Arnold, M. (2007). *Evolution Through Genetic Exchange*. Oxford University Press, New York.

Baldassarre, D.T. & Webster, M.S. (2013). Experimental evidence that extra-pair mating drives asymmetrical introgression of a sexual trait. *Proc. Roy. Soc. B-Biol. Sci.*, 280, 20132175.

Baldassarre, D.T., White, T.A., Karubian, J. & Webster, M.S. (2014). Genomic and morphological analysis of a semipermeable avian hybrid zone suggests asymmetrical introgression of a sexual signal. *Evolution*, 68, 2644–2657.

Böhme, W. (1986). Podarcis muralis (Laurenti, 1768) – Mauereidechse. In: *Handbuch der Reptilien und Amphibien Europas*, vol. 2 (ed. Grossenbacher, K.). Aula Verlag, Wiebelsheim, pp. 155–208.

Derryberry, E.P., Derryberry, G.E., Maley, J.M. & Brumfield, R.T. (2014). hzar: hybrid zone analysis using an R software package. *Mol. Ecol. Res.*, 14, 652–663.

Development Core Team, R. (2010). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, Available at: http://www.R-project.org. Last accessed 5 September 2015

Gay, L., Crochet, P.A., Bell, D.A. & Lenormand, T. (2008). Comparing clines on molecular and phenotypic traits in hybrid zones: a window on tension zone models. *Evolution*, 62, 2789–2806.

Grether, G.F., Anderson, C.N., Drury, J.P., Kirschel, A.N.G., Losin, N., Okamoto, K. *et al.* (2013). The evolutionary consequence of interspecific aggression. *Ann. NY Acad. Sci.*, 1289, 48–68.

Haldane, J.B.S. (1922). Sex ratio and unisexual sterility in hybrid animals. *J. Genet.*, 12, 101–109.

Heathcote, R.J.P. (2013). Secondary contact in the European wall lizard. PhD Thesis, University of Oxford, Oxford, UK.

Hedrick, P.W. (2013). Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Mol. Ecol.*, 22, 4606–4618.

Hubbs, C.L. (1955). Hybridisation between fish species in nature. *System. Zool.*, 4, 1–20.

Jakobsson, M. & Rosenberg, N.A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23, 1801–1806.

Marshall, T.C., Slate, J., Kruuk, L.E.B. & Pemberton, J.M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.*, 7, 639–655.

McDonald, D.B., Clay, R.P., Brumfield, R.T. & Braun, M.J. (2001). Sexual selection on pluymage and behaviour in an avian hybrid zone: experimental tests of male-male interactions. *Evolution*, 55, 1443–1451.

Michaelides, S., While, G.M., Bell, C. & Uller, T. (2013). Human introductions create opportunities for intra-specific hybridization in an alien lizard. *Biol. Invas.*, 15, 1101–1112.

Michaelides, S., While, G.M., Zajac, N. & Uller, T. (2015). Widespread primary, but geographically restricted secondary, human introductions of wall lizards, *Podarcis muralis*. *Mol. Ecol.*, 24, 2702–2714.

Mundy, N. (2005). A window into the genetics of evolution: MCR1 and plumage colouration in birds. *Proc. Roy. Soc. B-Biol. Sci.*, 272, 1633–1640.

Olsson, M. & Madsen, T. (1995). Female choice on male quantitative traits in lizards-why is it so rare? *Behav. Ecol. Sociobiol.*, 36, 179–184.

Olsson, M. & Madsen, T. (1998). Sexual selection and sperm competition in reptiles. In: *Sperm Competition and Sexual Selection* (eds Birkhead, T.R. & Møller, A.P.). Academic Press, London, pp. 504–570.

Olsson, M. & Shine, R. (1997). Advantages of multiple matings to females: a test of the infertility hypothesis using lizards. *Evolution*, 51, 1684–1688.

Pardo-Diaz, C., Salazar, C., Baxter, S.W., Merot, C., Figueiredo-Ready, W., Joron, M. *et al.* (2012). Adaptive introgression across species boundaries in heliconius butterflies. *PLoS Genet.*, 8, e1002752.

Parsons, T.J., Olson, S.L. & Braun, M.J. (1993). Unidirectional spread of secondary plumage traits across an avian hybrid zone. *Science*, 260, 1643–1646.

Peakall, R. & Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 28, 2537–2539.

Pearson, S.F. (2000). Behavioral symmetries in a moving hybrid zone. *Behav. Ecol.*, 11, 84–92.

Peason, S.F. & Rohwer, S. (2000). Asymmetries in male aggression across an avian hybrid zone. *Behav. Ecol.*, 11, 93–101.

Petit, R.J. & Excoffier, L. (2009). Gene flow and species delimitation. *Trends Ecol. Evol.*, 24, 386–393.

Pfennig, K. (2007). Facultative mate choice drives adaptive hybridization. *Science*, 318, 965–967.

Prado, F., Timothy, B. & Kerr, B. (2009). Introgression of sexually selected traits in lek-mating species. *Evol. Ecol. Res.*, 11, 1235–1250.

Price, T.D. (2008). *Speciation in Birds*. Roberts and Company Publishers, Greenwood Village.

Pritchard, J., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.

Sacchi, R., Pupin, F., Gentilli, A., Rubolini, D., Scali, S., Fasola, M. *et al.* (2009). Male-Male combats in a polymorphic lizard: residency and size, but not color, affect fighting rules and contest outcome. *Aggressive Behav.*, 35, 274–283.

Saetre, G.-P., Moum, T., Bures, S., Kral, M., Adamjan, M. & Moreno, J. (1997). A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature*, 387, 589–592.

Salvi, D., Harris, D.J., Kaliontzopoulou, A., Carretero, M.A. & Pinho, C. (2013). Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard. *BMC Evol. Biol.*, 13, 147.

Schulte, U., Hochkirch, A., Loetters, S., Roedder, D., Schweiger, S., Weimann, T. *et al.* (2012a). Cryptic niche conservatism among evolutionary lineages of an invasive lizard. *Global Ecol. Biogeogr.*, 21, 198–211.

Schulte, U., Veith, M. & Hochkirch, A. (2012b). Rapid genetic assimilation of native wall lizard populations (Podarcis muralis) through extensive hybridization with introduced lineages. *Mol. Ecol.*, 21, 4313–4326.

Seehausen, O., Terai, Y., Magalhaes, I.S., Carleton, K.L., Mrosso, H.D.J., Miyagi, R. *et al.* (2008). Speciation through sensory drive in cichlid fish. *Nature*, 455, 620–626.

Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F.R., Shih, C.H. *et al.* (2011). Adaptive introgression of anticoagulant rodent poison resistance by hybridization between old world mice. *Curr. Biol.*, 21, 1296–1301.

Stein, A.C. & Uy, J.A.C. (2006). Unidirectional introgression of a sexually selected trait across an avian hybrid zone: a role for female choice? *Evolution*, 60, 1476–1485.

Szymura, J.M. & Barton, N.H. (1986). Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *Bombina variegata*, near cracow in southern Poland. *Evolution*, 40, 1141–1159.

Teeter, K.C., Payseur, B.A., Harris, L.W., Bakewell, M.A., Thibodeau, L.M., O'Brien, J.E. *et al.* (2007). Genome-wide patterns of gene flow across a house mouse hybrid zone. *Genome Res.*, 18, 67–76.

Uy, J.A.C. & Stein, A.C. (2007). Variable visual habitats may influence the spread of colourful plumage across an avian hybrid zone. *J. Evol. Biol.*, 20, 1847–1858.

Vallin, N., Rice, A.M., Bailey, R.I., Husby, A. & Qvarnstrom, A. (2012). Positive feedback between ecological and reproductive character displacement in a young avian hybrid zone. *Evolution*, 66, 1167–1179.

Veen, T., Borge, T., Griffith, S.C., Saetre, G.-P., Bures, S., Gustafsson, L. *et al.* (2001). Hybridisation and adaptive mate choice in fly catchers. *Nature*, 411, 45–50.

Whitney, K.D., Randell, R.A. & Rieseberg, L.H. (2006). Adaptive introgression of herbivore resistance traits in the weedy sunflower Helianthus annuus. *Am. Nat.*, 167, 794–807.

Whitney, K.D., Randell, R.A. & Rieseberg, L.H. (2010). Adaptive introgression of abiotic tolerance traits in the sunflower Helianthus annuus. *New Phytol.*, 187, 230–239.

Wirtz, P. (1999). Mother species-father species: unidirectional hybridization in animals with female choice. *Anim. Behav.*, 58, 1–12.

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