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Experimental contact zones reveal causes and targets of sexual selection in hybridizing lizards

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Summary

1. Divergence in sexually selected traits in allopatry should affect the degree and direction of hybridization. However, few studies have established the causes and targets of sexual selection during secondary contact.

2. Common wall lizards (*Podarcis muralis*) from north-central Italy have highly exaggerated male sexual traits compared to populations in Western Europe. Using experimental populations, we show that this creates asymmetries in male dominance, spatial habitat use and reproductive success upon secondary contact. Hybridization occurred almost exclusively between males of the Italian lineage and females of the Western European lineage.

3. We provide evidence to suggest stronger ongoing selection on male sexual traits within the dominant Italian lineage. However, these same characters did not predict hybridization, and hybrid matings contributed little to variance in male reproductive success. Instead, most hybrid offspring were sired by Italian males displaying phenotypes associated with lower within-lineage reproductive success.

4. Thus, highly directional hybridization arises because some Italian males are out-competed within their own lineage but remain competitive relative to males of the other lineage.

5. This pattern of hybridization is consistent with the direction of introgression in natural contact zones, but our data suggest that sexual selection acting through hybridization may be weak at the leading edge of natural hybrid zones.

Key-words: behaviour, hybridization, introgression, male–male competition, *Podarcis*

Introduction

Divergence in behavioural or morphological traits whilst populations are in allopatry can restrict gene flow between closely related lineages upon secondary contact (Coyne & Orr 2004). Genetic analyses of hybrid zones often, however, reveal directional patterns of introgression (e.g. Singhal & Moritz 2013; Johnson *et al*. 2015). Sexual selection should be of particular importance within this context because the strength and direction of gene flow will depend upon mating behaviour and the propensity of individuals from each lineage to interact and hybridize in zones of secondary contact (e.g. Willis, Ryan & Rosenthal 2011; Charpentier *et al.* 2012). Pre-copulatory behaviours and morphology associated with mate acquisition and fertilization success can evolve rapidly under sexual selection and often show greater divergence among lineages than non-sexual characteristics (Panhuis *et al.* 2001; Mendelson & Shaw 2005). Consequently, the extent to which divergent sexual characteristics favour within- and between-lineage reproductive success can mediate patterns of hybridization. When one lineage has evolved advantageous sexual characteristics over the other, sexual selection may then act as the main driving force for genetic and phenotypic introgression (e.g. sexually selected introgression; Stein & Uy 2006).

Most existing studies of sexually selected introgression have implicated female choice as the main driver of gene flow (e.g. Stein & Uy 2006; Baldassarre & Webster 2013). However, male–male competition can overcome the effects

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of female mate preferences (e.g. Reichard *et al*. 2005) and cause extensive hybridization between taxa (Hartman *et al*. 2012; While *et al*. 2015). Therefore, in species where males defend territories and compete for access to females, divergence in traits that influence the outcome of male–male competition could have consequences for which individuals are more likely to engage in hybridization and determine the phenotypic targets of selection (e.g. Pearson & Rohwer 2000; Dijkstra & Groothuis 2011). Despite this, surprisingly few studies have sought to quantify sexual selection upon secondary contact.

Previous work on the common wall lizard, *Podarcis muralis* (Laurenti 1768), has documented asymmetric gene flow between two lineages, across several regions of secondary contact (While *et al*. 2015). The lineages, from the Italian Peninsula and from Western Europe, share a pattern of gene flow consistent with sexually selected introgression. Previous work also suggests that female choice based on male quantitative traits is absent or weak in this species (Heathcote *et al*. 2014), with no evidence that females of Western European or Italian origin discriminate between males of either lineage (Heathcote *et al*. 2016). This makes *P. muralis* a useful model for testing the role of sexual selection via male–male competition as a mediator of the strength and direction of hybridization. Here, we analyse data from experimental contact zones in outdoor enclosures to assess how phenotypic divergence between the lineages in male sexual traits causes asymmetric hybridization. We then assess the implications that this has for the strength and targets of sexual selection upon secondary contact.

Materials and methods

STUDY ANIMALS

Common wall lizards, *Podarcis muralis,* are small [48–75 mm snout-vent length (SVL)], diurnal, lacertids that are native to southern and central Europe. This species is strongly associated with human modified habitat (e.g. dry stone walls) and occupies a large geographic range (Schulte 2008; Salvi *et al*. 2013; While *et al*. 2015). Intraspecific diversity is high with several genetically and geographically distinct mitochondrial clades described (Giovannotti, Nisi-Cerioni & Caputo 2010; Schulte *et al*. 2012a; Salvi *et al*. 2013). The lineages that form the focus of this study represent two major mitochondrial clades that diverged approximately 2 million years ago (Gassert *et al*. 2013). Hereafter, animals referred to as from the Western European lineage fall within the western France subclade and animals referred to as from the Italian lineage fall within the Tuscan haplotype clade (*sensu* Schulte *et al*. 2012a). The lineages differ in morphology (see below and Fig. 1) and are often described as separate subspecies (Böhme 1986).

We captured 128 sexually mature lizards (>48 mm SVL) in April 2013, from three localities in Tuscany, northern Italy [Prato (43°54′N, 11°06′E), Greve di Chianti (43°35′N, 11°19′E) and Colle di Val D'Elsa (43°25′N, 11°06′E)], and four localities in western France [Dinan (48°27′N, 2°02′W), Josselin (47°57′N, 2°32′W), Pontchateau (47°26′N, 2°05′W) and Pouzagues (46°47′N, 0°50′E)]. Upon capture, we sexed and toe-clipped each lizard for unique identification and measured four body-size-related morphological traits (SVL, Mass, Head Width and Head Length). We removed ~5 mm of tail tip tissue from every individual, which we preserved in 90% ethanol. Two authors (GMW and TU) gave each lizard a dorsal greenness score (Greenness) from 1 to 10 (1 being pure brown, 10 being pure green; correlation between observer scores = 0·98). One author (GMW) photographed all individuals on their ventral and lateral sides using a Canon EOS 350D digital camera. From the photographs, we quantified ventral blackness coloration (Blackness) and the area of the outer ventral scales (OVS) with blue coloration (OVS Blue Area).

Fig. 1. Images of two male *Podarcis muralis* to show the typical Italian phenotype from north-central Italy (above) and Western European phenotype (below). Photographs by Ben Halliwell and Guillem Pérez i de Lanuza.

We transported the lizards from the field in cloth bags (kept below 10 °C) to laboratory facilities at the Department of Zoology, University of Oxford, UK. There, we housed the lizards in plastic terraria (590 × 390 × 415 mm) under a 12:12 light/dark cycle and provided them with 6 h of UV lighting per day. Each terrarium contained a 60-W heat lamp, sand substrate, a brick basking site and shelter. Most females were fecund with their first clutch of the breeding season at time of capture (wall lizards lay up to three clutches per year). We kept the females that had not ovulated at capture (assessed using palpation, e.g. Gartrell *et al*. 2002) with a male during their receptive phase and all other lizards were kept individually. All females laid their first clutch in the laboratory prior to commencing the experiment.

Upon establishment in the laboratory, one author (GPL) objectively measured four chromatic traits from each male (OVS Hue, OVS UV Chroma, Dorsal Hue and Dorsal Green Chroma) using a USB-2000 portable Ocean Optics diode-array spectrometer and a PX-2 xenon strobe light source (Pérez i de Lanuza, Carazo & Font 2014). We also measured maximum bite force (Bite Force) for all males and females, and mean testes mass for all males (Testes Mass), the latter of which was carried out at the completion of the experiment. See Appendix S1 (Supporting Information) for expanded details on the quantification of all morphological traits.

EXPERIMENTAL ENCLOSURES AND BEHAVIOURAL DATA COLLECTION

We simulated the initial stage of secondary contact by releasing lizards into eight (~7 × 7 m) experimental enclosures at the John Krebs Field Station, University of Oxford. The climate in Oxford falls within the variation in the non-native range of wall lizards in England. We note that this study may be most representative of secondary contact zones in England, which are the result of introductions (Michaelides *et al.* 2015).

Within each enclosure, we created a gradient in habitat complexity by constructing three types of sites that varied in structural complexity and the opportunity for thermoregulation. Each site consisted of two stacked pallets (1·14 m^2) sandwiched with a sheet of felt underlay, but varied in the number and construction of concrete breezeblocks placed above the pallets, which acted as both a shelter and a thermal resource. We arranged high-, medium- and low-quality pallets in a three-by-three organization from one side of the enclosure to the other (Fig. S1).

At the start of the experiment, we released 64 male lizards: four Italian (ITA) and four Western European (WEUR) males per enclosure. We monitored these males within their enclosures for at least 9 days whilst they established territories. We then released 64 females: four Italian and four Western European females per enclosure. With the exception of three females (added 1–3 days after), we released all female lizards into an enclosure simultaneously (see Appendix S1 for further details on assignment to enclosures). Prior to release, we marked all lizards for identification at a distance with a unique number on their dorsal side using a non-toxic, non-hypoallergenic marker pen (Mitsubishi Pencil Company Ltd., Tokyo, Japan).

Two authors (HEAM and JB) monitored the eight enclosures during May and June 2013 to collect positional and interaction data (see Appendix S1 & Table S2). This resulted in records of 5638 positional and 1138 social interaction observations. From the social interaction data, we classified 492 male–male interactions, 464 of which were deemed competitive, and 684 male–female interactions including 296 courtships and 65 matings. We retained competitive interactions, courtships and matings for analyses.

At the end of female gestation, we returned the lizards to laboratory facilities where females oviposited. We lost 15 female clutches from mortality (two ITA, two WEUR), failure of the female to reproduce (10 WEUR) or failure to recapture (one ITA) but were able to obtain reproductive output for two dead females via dissection. Western European females often produce only one seasonal clutch in the native range so an absence of second clutch production by ten WEUR females was not surprising. For the remaining females, we retrieved and counted the number of eggs within each clutch, and noted the presence and number of infertile eggs (assessed based on absence of calcified egg shell following Olsson & Shine 1997). Two Italian females produced fully infertile clutches, and a further ten eggs from five females (three ITA and two WEUR) were infertile or dumped but we included these when testing for differences in the potential reproductive output (i.e. clutch size) of Italian and Western European females.

We weighed each clutch and incubated fertile eggs at a constant 28 °C and humidity (5:1 vermiculite:water volume) until hatching. At hatching, we obtained tail tissue samples from all juveniles for paternity analysis, which were preserved in 90% ethanol. Average hatching success of offspring was 96% for Italian and 93% for Western European females, respectively. For ten of twelve aborted offspring, we successfully extracted DNA and assigned paternity.

PATERNITY ANALYSIS

We isolated DNA from all adults and 203 offspring (hatchlings: 191, embryos: 12) using the DNeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA, USA), following manufacturer's instructions (with overnight lysis). Given the limited number of potential fathers (eight per enclosure), we genotyped individuals at six microsatellite loci (Heathcote, Dawson & Uller 2015; Table S3 & Appendix S1). We assigned offspring paternity using CERVUS 3.0 (Marshall *et al.* 1998). Twenty offspring (18 hatchlings and two embryos) could not be reliably assigned a father because they amplified at fewer than three loci or mismatched within their mother–father–offspring trio at more than one locus. This resulted in the retainment of 183 offspring for further analyses.

STATISTICAL ANALYSES

All statistical analyses unless otherwise stated were carried out in R 3.1.2 (Core Team 2014). We ran linear mixed models (LMMs) and generalized linear mixed models (GLMMs) for phenotypic, spatial and behavioural analyses, including enclosure as a random effect when appropriate.

Spatial analyses

Spatial analyses were conducted in Ranges 8 (Kenward *et al.* 2008). We estimated home range areas from positional observations using a fixed-kernel contour analysis with a fixed smoothing parameter of 0·75 (Kie 2013; Table S4). We calculated home range size, percentage overlap and, for males, the number of overlapping females, at both the 50% (core home range) and 95% (total home range) isopleth level (Worton 1989). We assigned each lizard to a pallet quality based on the location where the kernel estimate indicated peak density. We used the spread of the location distribution (the grand mean of distances between locations; Spencer & Barrett 1984) of each male's positional observations as an indicator of the extent to which males defend a territory (e.g. Morrison, Keogh & Scott 2002). We tested for lineage differences in home range area, male–female overlap, habitat quality and spread. See Appendix S1 for expanded details.

Social, behavioural and genetic network analyses

To determine whether social interactions and spatial distribution could mediate hybridization between the two lineages, we ran Mantel permutation analyses on behavioural, spatial and genetic association networks in the compiled version of SOCPROG 2.4 (Whitehead 2009). First, for each enclosure, we tested for within-lineage assortativity in male–male competitive interactions, male–female courtships, observed matings and paternity using social networks weighted by the total number of observed interactions (or for paternity, the number of offspring) between each dyad. Secondly, we tested for significant correlations between these behavioural networks and core home range overlap (weighted by % overlap at the 50% isopleth) and paternity (weighted by numbers of offspring sired), respectively. All Mantel permutation analyses were based on 1000 permutations, which achieved stability in *P*-values. For each set of analyses, we combined the *P*-values for each enclosure into a single test statistic using Fisher's method (Fisher 1932).

Behavioural analyses

We calculated each male's dominance score (Dominance) based on David's method (David 1988), corrected for the numbers of interactions between dyads. We tested for significant differences between the lineages in Dominance with a LMM that included Lineage and SVL as fixed effects. The robustness of this result was confirmed through comparisons against randomized data sets, obtained via a Quadratic Assignment Procedure, based on 10 000 permutations of dominance scores per enclosure (Permute package, Simpson 2015).

Male reproductive success

We calculated the reproductive success of each male in terms of fertilization success (the total number of offspring sired) and mating success (the total number of female clutches including sired offspring) based on the paternity analysis. We excluded the use of behavioural observations of mating from the calculation of male mating success because of the potential for observation biases within and between the lineages. We examined male reproductive success separately by lineage because of differences between Italian and Western European females in the number of clutches produced, and the incidence of hybridization (see Results). Since the evolutionary consequences of selection will depend on relative rather than absolute reproductive success (Kingsolver & Pfennig 2007), we divided the fitness measures for each male by the mean for all males within his enclosure that were of the same lineage to generate relative measures of mating success and fertilization success for each male. The mean fitness values within each enclosure were calculated with the inclusion of non-siring/unmated males (Shuster 2009).

Estimates of the strength and targets of sexual selection

We quantified the contribution of variance in relative within-lineage (W) and between-lineage (B) fertilization success to overall variance in male fertilization success following Webster *et al.* (1995). We use this as a proxy for their relative contribution to selection on male sexual traits. To quantify the relative strength of pre- and post-copulatory sexual selection, we further partitioned W and B into the (co)variance contributions of male mating success (M), mate fecundity (N) and paternity share (P) (Webster *et al.* 1995). In addition, we characterized the strength of pre-copulatory sexual selection on males with the Bateman gradient (β_{SS}), the slope of the least squares regression of relative mating success on relative fertilization success (Jones 2009). We compared Bateman gradients between lineages using a LMM with relative mating success, lineage and their interaction as fixed effects, and enclosure as a random effect.

To identify the potential phenotypic targets for ongoing selection in Italian and Western European males, and to estimate the strength and direction of associations between traits and reproductive success, we performed multiple linear regression analyses with relative within-lineage fertilization success as the response variable and standardized (within-lineage: mean = 0, SD = 1) morphological traits and Dominance as fixed effects (Lande & Arnold 1983). To quantify the associations between Italian male traits and hybridization, we performed the same analyses with relative between-lineage fertilization success as the response variable. The low incidence of hybridization involving Western European males precluded similar analyses (see Results). We collapsed SVL, Head Length, Head Width, and Body Mass into a single principle component (PC1_BodySize, Table S5). Furthermore, Dorsal Hue and Dorsal Green Chroma were replaced with the greenness score, which was highly correlated with both traits (Dorsal Hue: $r = -0{\cdot}88$, Dorsal Green Chroma: $r = 0{\cdot}89$). To avoid over parameterization of our models, we performed the regression analyses on the nine remaining phenotypic traits separately for body size and performance related traits (Dominance, PC1_BodySize, Bite Force, Testes Mass) and coloration traits (Greenness, Blackness, OVS Blue Area, OVS Hue, OVS UV Chroma). We ran and evaluated all candidate models (Table S6, including single explanatory variables) based on the second-order Akaike Information Criterion (AICc) and selected the top performing models as those <2 ΔAICc from the best approximating model (Burnham & Anderson 2002). We report full/partial regression coefficients for traits in the top performing models and parameter estimates based on full-model averaging, that is with shrinkage (Symonds & Moussalli 2011).

We supported our findings from multiple regression analyses by calculating standardized linear regression coefficients (β_i) from single-trait models controlling for SVL. In addition, because associations between male phenotypic traits and reproductive success may be nonlinear in form, we estimated standardized quadratic regression coefficients (γ_{ii}) as twice the coefficient for the second-order term from models including both linear and quadratic terms (Stinchcombe *et al.* 2008). We did not test for significant cross-product terms (i.e. correlational selection) to avoid over-fitting of the models relative to sample size.

Results

MORPHOLOGICAL AND SPATIAL ASYMMETRIES BETWEEN THE LINEAGES

Italian males had exaggerated phenotypes compared to Western European males, and several characters suggested to be under sexual selection in *Podarcis* lizards (e.g. Sacchi *et al.* 2009; Huyghe *et al.* 2012) showed greater sexual dimorphism in the Italian lineage (Table 1, Table S1).

Males had larger core and total home ranges than females, but there were no significant differences between the lineages (Tables S4 and S8). Male core home ranges were not evenly distributed across habitat qualities, and most males occupied either the high- or low-quality end of each enclosure (High ($n = 29$), Medium ($n = 9$), Low ($n = 25$): $\chi^2 = 10{\cdot}66$, $P = 0{\cdot}004$), with the most dominant Italian males occupying high-quality sites (GLMM (Binomial) for Male Habitat Quality: Lineage: $\chi^2 = 6{\cdot}95$, $P = 0{\cdot}008$, Dominance: $\chi^2 = 0{\cdot}18$, $P = 0{\cdot}67$, Lineage × Dominance: $\chi^2 = 6{\cdot}16$, $P = 0{\cdot}01$). By contrast, female core home ranges were evenly distributed across habitat qualities (High ($n = 19$), Medium ($n = 21$), Low ($n = 24$): $\chi^2 = 0{\cdot}59$, $P = 0{\cdot}74$). Consequently, there were no differences in male–females overlap between the lineages (Table S8). Italian males had similar clustering of observations regardless of the position of their core home range, whereas Western European males showed greater spread when the centre of their home range was a low-quality site (Origin: $F_{1,43} = 10{\cdot}7$, $P < 0{\cdot}001$, Habitat Quality: $F_{1,44} = 1{\cdot}00$, $P = 0{\cdot}32$: Origin × Habitat Quality: $F_{1,45} = 3{\cdot}79$, $P = 0{\cdot}06$).

BEHAVIOURAL ASYMMETRIES BETWEEN THE LINEAGES

Male–male competitive interactions were not assortative by lineage ($\chi^2 = 19{\cdot}29$, $P = 0{\cdot}24$, d.f. = 16). Italian males were significantly more dominant than Western European males [ITA Males: $4{\cdot}10 \pm 0{\cdot}13$, WEUR Males: $2{\cdot}81 \pm 0{\cdot}08$, Lineage: $F_{1,53} = 60{\cdot}87$, $P < 0{\cdot}001$ SVL: $F_{1,56} = 5{\cdot}84$, $P = 0{\cdot}019$, (QAP: Lineage: $P < 0{\cdot}001$, SVL: $P = 0{\cdot}05$)], and dominance was more strongly correlated with body size in the Italian lineage than in the Western European lineage (Table S9).

Italian males courted more females of both origins (ITA Females Courted: Lineage: $\chi^2 = 26{\cdot}50$, $P < 0{\cdot}001$, SVL: $\chi^2 = 0{\cdot}39$, $P = 0{\cdot}53$; WEUR Females Courted: Lineage: $\chi^2 = 20{\cdot}22$, $P < 0{\cdot}001$, SVL: $\chi^2 = 1{\cdot}57$, $P = 0{\cdot}21$) and, for Italian but not Western European males, Dominance was a strong predictor of both number of females courted (ITA Males: Dominance: $\chi^2 = 12{\cdot}17$, $P < 0{\cdot}001$, WEUR

Table 1. Results from linear mixed models examining divergence and sexual dimorphism in body size, performance and coloration between Italian and Western European lizards. A covariate, SVL, Head Length (HL) or Mass (M), was included in the models when appropriate, and population of origin nested within lineage was included as a random effect

Response variable	Lineage	Sex	Lineage × Sex	Covariate
SVL	$F_{1,4} = 0{\cdot}00$, $P = 0{\cdot}95$	$F_{1,123} = 5{\cdot}28$, $P = 0{\cdot}02$	$F_{1,122} = 5{\cdot}21$, $P = 0{\cdot}02$	
Head Length	**$F_{1,4} = 38{\cdot}10$, $P < 0{\cdot}002$**	**$F_{1,123} = 850{\cdot}78$, $P < 0{\cdot}001$**	$F_{1,121} = 1{\cdot}85$, $P = 0{\cdot}18$	**SVL: $F_{1,123} = 291{\cdot}56$, $P < 0{\cdot}001$**
Head Width	**$F_{1,4} = 101{\cdot}63$, $P < 0{\cdot}001$**	**$F_{1,123} = 291{\cdot}63$, $P < 0{\cdot}001$**	$F_{1,122} = 0{\cdot}99$, $P = 0{\cdot}32$	**SVL: $F_{1,121} = 187{\cdot}08$, $P < 0{\cdot}001$**
Mass	$F_{1,5} = 12{\cdot}23$, $P = 0{\cdot}02$	**$F_{1,123} = 43{\cdot}69$, $P < 0{\cdot}001$**	$F_{1,121} = 0{\cdot}39$, $P = 0{\cdot}54$	**SVL: $F_{1,123} = 227{\cdot}95$, $P < 0{\cdot}001$**
Bite Force	$F_{1,6} = 5{\cdot}72$, $P = 0{\cdot}04$	$F_{1,115} = 1{\cdot}03$, $P = 0{\cdot}31$	**$F_{1,115} = 10{\cdot}02$, $P = 0{\cdot}002$**	**HL: $F_{1,114} = 47{\cdot}40$, $P < 0{\cdot}001$**
Testes Mass	$F_{1,5} = 15{\cdot}47$, $P = 0{\cdot}01$			**M: $F_{1,56} = 12{\cdot}74$, $P < 0{\cdot}001$**
Dorsal Hue	**$F_{1,4} = 163{\cdot}66$, $P < 0{\cdot}001$**			SVL: $F_{1,48} = 0{\cdot}06$, $P = 0{\cdot}81$
Dorsal Green Chroma	**$F_{1,4} = 177{\cdot}72$, $P < 0{\cdot}001$**			SVL: $F_{1,48} = 1{\cdot}57$, $P = 0{\cdot}22$
Blackness	**$F_{1,5} = 25{\cdot}80$, $P = 0{\cdot}004$**	**$F_{1,121} = 47{\cdot}44$, $P < 0{\cdot}001$**	$F_{1,120} = 1{\cdot}25$, $P = 0{\cdot}27$	**SVL: $F_{1,121} = 10{\cdot}21$, $P = 0{\cdot}002$**
OVS Blue Area	**$F_{1,4} = 33{\cdot}76$, $P = 0{\cdot}004$**	**$F_{1,123} = 73{\cdot}64$, $P < 0{\cdot}001$**	$F_{1,121} = 0{\cdot}77$, $P = 0{\cdot}38$	SVL: $F_{1,123} = 4{\cdot}44$, $P = 0{\cdot}04$
OVS Hue	$F_{1,4} = 7{\cdot}70$, $P = 0{\cdot}05$			SVL: $F_{1,47} = 0{\cdot}53$, $P = 0{\cdot}47$
OVS UV Chroma	**$F_{1,4} = 67{\cdot}59$, $P = 0{\cdot}001$**			SVL: $F_{1,43} = 1{\cdot}87$, $P = 0{\cdot}18$

Results for main effects are reported from models excluding non-significant interaction terms. Significant effects are highlighted in bold based on a threshold of $\alpha \leq 0{\cdot}004$, adjusted from the nominal $\alpha < 0{\cdot}05$ following Bonferroni correction for the number of tests performed on these data.

Males: Dominance: $\chi^2 = 0{\cdot}47$, $P = 0{\cdot}49$) and number of courtships (ITA Males: Dominance: 93·69, $P < 0{\cdot}001$, WEUR Males: Dominance: $\chi^2 = 0{\cdot}51$, $P = 0{\cdot}47$). Overall, courtship networks were significantly assortative across enclosures ($\chi^2 = 28{\cdot}28$, $P = 0{\cdot}03$, d.f. = 16). Consequently, Italian females received more courtships on average than Western European females, and larger females received more courtships than smaller females (Female Lineage: $\chi^2 = 11{\cdot}40$, $P < 0{\cdot}001$, Female SVL: $\chi^2 = 13{\cdot}96$, $P < 0{\cdot}001$). Sixty five of the 296 observed courtships resulted in an observed mating. In contrast to courtships, we found no assortativity by lineage in observed matings across enclosures ($\chi^2 = 9{\cdot}0$, $P = 0{\cdot}90$, d.f. = 16).

REPRODUCTIVE SUCCESS

There were no significant differences in clutch size between Italian and Western European females (Table 2, ITA: 4·81 ± 0·24, WEUR: 4·25 ± 0·32). Overall, the incidence of multiple paternity was higher for Western European females (ITA Clutches: 71% and WEUR Clutches: 85%), but there was no significant difference between the lineages in the number of fathers per clutch after controlling for SVL and clutch size (Table 2, ITA Clutches: 2·04 ± 0·20, WEUR Clutches: 2·50 ± 0·18). Italian males sired significantly more offspring than Western European males (Lineage: $\chi^2 = 21{\cdot}16$, $P < 0{\cdot}001$), but paternity was biased towards females of the same lineage ($\chi^2 = 60{\cdot}04$, $P < 0{\cdot}001$, d.f. = 16), and strongly predicted by both courtship networks ($\chi^2 = 69{\cdot}57$, $P < 0{\cdot}001$, d.f. = 16), and the percentage overlap in core home ranges between males and females ($\chi^2 = 35{\cdot}27$, $P < 0{\cdot}001$, d.f. = 16). Thirty-four offspring were identified as hybrids, and the direction of hybridization was highly asymmetric (Table 2). Of the 104 offspring produced by ITA females 98 were sired by ITA males and six by WEUR males. Of the 79 offspring produced by WEUR females 51 were sired by WEUR males and 28 by ITA males.

Table 2. Results from generalized linear mixed models testing for lineage differences in clutch size, fathers per clutch and proportion hybrid offspring. The effects of lineage on clutch size and fathers per clutch were tested with GLMMs (Poisson error)

Response variable	Lineage	SVL	Clutch size
Clutch size	$\chi^2 = 1{\cdot}60$, $P = 0{\cdot}21$	$\chi^2 = 2{\cdot}38$, $P = 0{\cdot}12$	
Fathers per clutch	$\chi^2 = 1{\cdot}18$, $P = 0{\cdot}28$	$\chi^2 = 0{\cdot}64$, $P = 0{\cdot}42$	$\chi^2 = 0{\cdot}88$, $P = 0{\cdot}35$
Proportion hybrid offspring	**$\chi^2 = 28{\cdot}65$, $P < 0{\cdot}001$**	$\chi^2 = 0{\cdot}49$, $P = 0{\cdot}48$	

The effect of lineage on proportion hybrid offspring was tested with a GLMM (binomial error). Female SVL was included as a covariate in all models and clutch size as a covariate when analysing fathers per clutch. Significant effects are highlighted in bold.

VARIANCE IN MALE REPRODUCTIVE SUCCESS AND ASYMMETRIES IN SEXUAL SELECTION

Within-lineage fertilization success was by far the greatest contributor to variance in male reproductive success with hybrid offspring responsible for <10% of the overall variance in male reproductive success in both lineages. For both lineages, variance in mating success (M) contributed most to overall variance in within-lineage fertilization success. Paternity share (P) made a 14% larger contribution to variance in success for Italian males than for Western

European males. For both lineages, the contributions of mate fecundity (N) and the covariance among components were low (see Table S13 for variance contributions in full).

Males of both lineages had strong, positive Bateman gradients (ITA β_{SS}: 1·45, $CI_{95\%}$ = 1·13, 1·77; WEUR β_{SS}: 1·02, $CI_{95\%}$ = 0·79, 1·26) but the gradient was stronger for Italian males (Relative Mating Success: $F_{1,52}$ = 123·71, P < 0·001, Lineage: $F_{1,52}$ = 3·48, P = 0·07, Lineage × Relative Mating Success: $F_{1,52}$ = 5·03, P = 0·03). For Italian males, within-lineage reproductive success was best explained by a model including Dominance and Testes Mass, and from the coloration traits, a model including OVS Hue and OVS UV Chroma (Table 3, see Tables S10–S12 for model selection tables in full). These conclusions were supported by single-trait models suggesting directional selection on Dominance, Testes Mass and OVS Hue, in addition to disruptive selection on Greenness and OVS Blue Area (Table S14). For Western European males, five models with equal support suggested positive directional selection on body size and performance traits (Table 3; PC1_BodySize has negative factor loadings, Table S5). Only Ventral Blackness (positive coefficient) and OVS UV Chroma (negative coefficient) were retained from multiple regression analyses on Western European coloration traits, but the null model was equally well supported (Table 3). The conclusion that directional selection on the coloration of Western European males is weak was supported by single-trait models (Table S14).

For between-lineage fertilization success of Italian males, the best supported models for coloration suggested the opposite direction of associations with OVS UV Hue (positive coefficient) and Greenness (negative coefficient); however, the null model was equally well supported (Table 4). Similarly, single-trait models for between-lineage fertilization success indicated a general trend for reversed direction when compared to within-lineage fertilization success (Fig. 2, Table S14).

Discussion

Phenotypic divergence is typically expected to reduce the likelihood of hybridization between taxa in sympatry (Coyne & Orr 2004). However, this may not apply in contact zones between lineages that are in intermediate stages of divergence (Coyne & Orr 1989). In fact, rather than limit gene exchange, phenotypic differences in sexually selected traits may actively promote hybridization in a given direction (e.g. Parsons, Olson & Braun 1993; Baldassarre & Webster 2013). Experimental demonstration of highly asymmetric hybridization between lineages of the common wall lizard, *Podarcis muralis,* is consistent with historical differences in the strength of sexual selection, which makes males of one lineage competitively superior (While *et al.* 2015). However, our results reveal that most hybrid offspring were sired by males of the dominant lineage displaying traits associated with relatively low reproductive success with females of their own lineage. The results are consistent with the direction of introgression in regions of secondary contact (While *et al.* 2015), but, together with the finding that hybridization contributed little to variance in fertilization success, suggest that the strength of sexual selection operating through hybridization may be relatively weak at the leading edge of natural hybrid zones.

As predicted given the differences in male morphology and behaviour, Italian males were strongly dominant over Western European males and achieved greater reproductive success (over and above differences via fewer second clutches from Western European females). In addition, the within-lineage Bateman gradient was steeper, and selection on sexual traits was stronger, for Italian males. Consistent with the more pronounced sexual dimorphism in the Italian lineage, dominance and body coloration strongly predicted reproductive success for Italian but not for Western European males. Sexual dichromatism is positively correlated with sexual size dimorphism across a wide range of lacertid lizards and is probably driven by intrasexual selection (Pérez i de Lanuza, Font & Monterde 2013). Our data support that both UV and non-UV coloration act as sexual ornamentation in wall lizards. Moreover, our results suggest that there is likely to be stronger contemporary sexual selection on coloration traits in populations of the Italian lineage compared to the Western European lineage. However, some spectral variables of UV-blue outer ventral scales are also positively correlated with fighting ability and body condition in Western European males (Pérez i de Lanuza, Carazo & Font 2014). This implies that Western European males will respond to the same colour signals as Italian males, which gives Italian males an advantage in male–male competition. The higher reproductive success for males with larger testes may also point towards a competitive advantage for Italian males in sperm competition (Birkhead & Møller 1998).

In response to this competitive social environment, our space use and behavioural data suggests that some Western European males modify their behaviour and adopt a 'floater' strategy (Oliveira, Taborsky & Brockmann 2008). In contrast, the least dominant Western European males were apparently tolerated within the territories of the most dominant Italian males. Conditional behavioural tactics have been demonstrated in lizards (e.g. Noble *et al.* 2013). However, in our system, neither strategy appears to allow Western European males access to females of the opposite lineage, creating close to unidirectional hybridization (*sensu* Wirtz 1999). These results could predict that exaggerated sexually selected male traits will increase reproductive success with females of the opposite lineage. However, we show that selection on male quantitative traits through hybridization was weak, or even reversed in sign. This pattern could arise if females of the Western European lineage preferred subdominant Italian males (see Rosenthal 2013). Although we cannot completely exclude this explanation, previous work has shown that female discrimination of males with different quantitative characters is weak or

Table 3. Top supported models (<2 ΔAICc) from multiple regression analyses to assess the best phenotypic predictors of relative within-lineage fertilization success of Italian and Western European males. For each model, the number of parameters (K), the relative likelihood (AICcWt) and the evidence ratio with reference to the best approximating model (ER) are reported

Lineage	Analyses	Model	K	AICc	ΔAICc	AICcWt	ER	Trait	β	$CI_{95\%}$	Model-averaged β	Unconditional $CI_{95\%}$
ITA	Body Size and Performance (*n* = 29)	8	5	89·09	0	0·26	1·00	Dominance	0·33	−0·04 0·68	0·23	−0·25 0·72
								Testes Mass	0·42	0·06 0·78	0·39	−0·11 0·89
		5	4	89·30	0·21	0·24	1·11	Testes Mass	0·53	0·17 0·89		
		1	*3*	*94·09*	*5·00*	*0·02*	*12·18*	*Null*				
	Coloration (*n* = 30)	16	5	92·82	0	0·33	1·00	OVS Hue	−0·63	−1·03 −0·24	−0·48	−1·07 0·11
								OVS UV Chroma	−0·48	−0·87 −0·08	−0·31	−0·86 0·25
		1	*3*	*96·36*	*3·54*	*0·06*	*5·87*	*Null*				
WEUR	Body Size and Performance (*n* = 30)	5	4	89·46	0	0·22	1·00	Testes Mass	0·26	−0·06 0·60	0·13	−0·22 0·48
		2	4	90·60	1·14	0·12	1·77	Dominance	0·17	−0·15 0·54	0·08	−0·21 0·36
		3	4	90·74	1·28	0·11	1·90	PC1_BodySize	−0·18	−0·52 0·17	−0·05	−0·29 0·20
		8	5	90·80	1·34	0·11	1·95	Dominance	0·21	−0·12 0·55		
								Testes Mass	0·28	−0·06 0·61		
		4	4	90·94	1·48	0·10	2·10	Bite Force	0·16	−0·18 0·51	0·03	−0·19 0·25
		1	*3*	*94·31*	*4·85*		*11·33*	*Null*				
	Coloration (*n* = 24)	6	4	72·57	0	0·17	1·00	OVS UV Chroma	−0·34	−0·71 0·04	−0·17	−0·59 0·25
		1	3	72·75	0·18	0·16	1·09	Null				
		13	5	73·35	0·78	0·12	1·48	Blackness	0·28	−0·06 0·63	0·12	−0·25 0·49
								OVS UV Chroma	−0·33	−0·69 0·01		
		3	4	73·43	0·85	0·11	1·53	Blackness	0·29	−0·09 0·66		

When not included within the top supported models, AICc values for the null model are presented in italics for comparison. Regression coefficients (β) and bootstrap estimates of 95% confidence intervals ($CI_{95\%}$) are reported for all traits in the models. Model-averaged parameter estimates (model-averaged β) and unconditional 95% confidence intervals (unconditional $CI_{95\%}$) are also presented, generated via full-model averaging based on all candidate models.

Table 4. Top supported models (<2 ΔAICc) from multiple regression analyses to assess the best phenotypic predictors of relative between-lineage fertilization success of Italian males

Lineage	Analyses	Model	K	AICc	ΔAICc	AICcWt	ER	Trait	β	$CI_{95\%}$	Model-averaged β	Unconditional $CI_{95\%}$
ITA	Body Size and Performance (*n* = 29)	1	3	95·07	0	0·29	1·00	Null				
		5	4	96·74	1·67	0·13	1·88	Testes Mass	0·21	−0·18 0·61	0·14	−0·27 0·54
	Coloration (*n* = 30)	5	4	97·16	0	0·13	1·00	OVS Hue	0·35	−0·04 0·73	0·12	−0·27 0·51
		1	3	97·63	0·47	0·10	1·26	Null				
		10	5	97·75	0·59	0·09	1·35	Greenness	−0·36	−0·74 0·01	−0·16	−0·60 0·27
								OVS UV Chroma	−0·33	−0·71 0·05	−0·09	−0·44 0·26
		2	4	97·77	0·61	0·09	1·35	Greenness	−0·32	−0·70 0·08		
		9	5	98·24	1·08	0·07	1·71	Greenness	−0·26	−0·64 0·12		
								OVS Hue	0·30	−0·07 0·68		
		6	4	98·37	1·20	0·07	1·83	OVS UV Chroma	−0·28	−0·67 0·12		

For each model, the number of parameters (K), the relative likelihood (AICcWt) and the evidence ratio with reference to the best approximating model (ER) are reported. Regression coefficients (β) and bootstrap estimates of 95% confidence intervals ($CI_{95\%}$) are reported for all traits in the models. Model-averaged parameter estimates (model-averaged β) and unconditional 95% confidence intervals (unconditional $CI_{95\%}$) are also presented, generated via full-model averaging based on all candidate models.

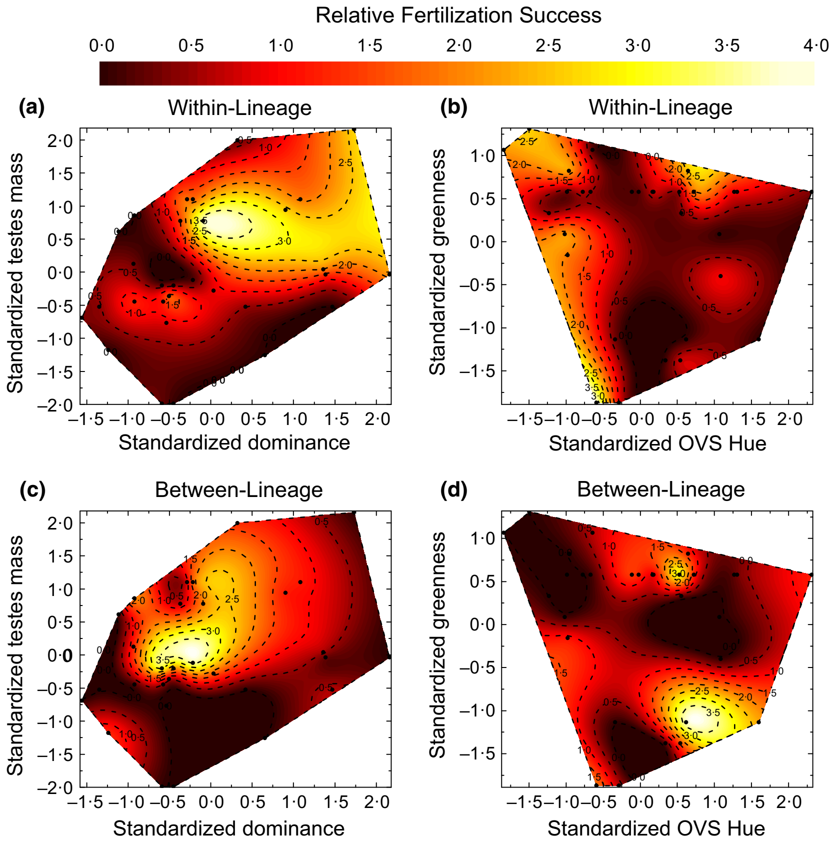

Fig. 2. Contour plots for Italian males depicting the relationships between four sexually selected traits [Dominance and Testes Mass, shown in (a) and (c), and Greenness and OVS UV Hue, shown in (b) and (d)] and relative within-lineage [(a) and (b)] or between- lineage [(c) and (d)] fertilization success (colour gradient: dark red = 0, light yellow = 4). Plots are shown to illustrate differences in the form and direction of associations when comparing within-lineage and between-lineage fertilization success. Contours were predicted by triangulation of the data points followed by linear interpolation.

absent (Heathcote *et al.* 2014), to the extent that females do not even discriminate between males of the two lineages (Heathcote *et al.* 2016). Therefore, these patterns of hybridization are more likely to be driven by males. Since courtships indicate that males prefer females of their own lineage, subdominant Italian males should be excluded from access to preferred, Italian, females and, therefore, more prone to hybridize. Asymmetric patterns of hybridization could then arise because the less dominant Italian males are still competitively superior over Western European males; which is supported by the dominance hierarchies in the enclosures. More generally, when males of different lineages recognize each other as competitors, as in wall lizards, asymmetric hybridization should be especially pronounced if one lineage has a consistent competitive advantage over the other (see also Pearson & Rohwer 2000; Rosenfield & Kodric-Brown 2003).

Combined, our results suggest that individuals with exaggerated sexual traits may not promote directional introgression at the leading edge of the hybrid zone. Despite this, previous work has documented directional nuclear gene flow from the Italian lineage into the Western European lineage and even greater introgression of sexual traits (head size, and dorsal and ventral coloration, While *et al.* 2015). There are several explanations that could account for the observed patterns of introgression despite our experimental findings. First, males with Italian phenotypes should have high rates of hybridization if the population is biased towards Western European individuals since encounter rates with preferred Italian females will be low. Hybridization involving dominant males could be further enhanced by a reduction in Italian male mate preferences in response to low encounters with Italian females (e.g. Willis, Ryan & Rosenthal 2011; Verzijden *et al.* 2012). Indeed, in non-native hybrid zones in Germany, the introduction of small numbers of Italian lizards has resulted in extensive introgression into native Western European populations (Schulte, Veith & Hochkirch 2012b; While *et al.* 2015). Furthermore, results from theoretical models (e.g. Currat & Excoffier 2005) suggest that introgression could be extensive, even when hybridization is limited, due to competitive displacement or in an expanding hybrid zone (Buggs 2007). Secondly, as the female population becomes more admixed, male mate preferences for their own lineage will no longer limit the overall reproductive success of males with exaggerated sexual traits. In addition, since subdominant males may be young rather than intrinsically low quality, the quantitative measures of their sexual characters at the time of hybridization may not represent their true breeding values for those traits (Pemberton 2010). Thus, hybrid male offspring could exhibit highly exaggerated sexual characters and be highly competitive even if sired by subdominant fathers. These potential influences make it difficult to predict how variation in the propensity to hybridize among Italian males will influence the broader geographic patterns of introgression in native and non-native regions of secondary contact. Detailed studies of phenotypic variation and selection at the leading edge of natural hybrid zones would be interesting in this regard.

In summary, our results highlight how behavioural interactions among individuals can shape hybridization. We demonstrated experimentally that asymmetries in male–male competitive ability are sufficient to promote asymmetric hybridization between lineages of wall lizards upon secondary contact, an initial step towards asymmetric gene flow. However, sexual selection on male traits through hybridization is likely to be weak at the leading edge of the hybrid zone. Whether, and how, this will influence the introgression of genetic and phenotypic characters requires further study.

Author contributions

HEAM, TU and GMW conceived and designed the study. HEAM coordinated the enclosure experiment and analysed the data. All authors contributed to data acquisition. HEAM, TU and GMW wrote the manuscript with substantial input from all authors.

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Data accessibility

Data deposited in the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.4pq11 (MacGregor *et al.* 2016).

References

Baldassarre, D.T. & Webster, M.S. (2013) Experimental evidence that extra-pair mating drives asymmetrical introgression of a sexual trait. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20132175.

Birkhead, T.R. & Møller, A.P. (1998) *Sperm Competition and Sexual Selection*. Academic Press, San Diego, CA, USA.

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

Buggs, R.J.A. (2007) Empirical study of hybrid zone movement. *Heredity*, **99**, 301–312.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York, NY, USA.

Charpentier, M.J.E., Fontaine, M.C., Cherel, E., Renoult, J.P., Jenkins, T., Benoit, L. *et al.* (2012) Genetic structure in a dynamic baboon hybrid zone corroborates behavioural observations in a hybrid population. *Molecular Ecology*, **21**, 715–731.

Coyne, J.A. & Orr, H.A. (1989) Patterns of speciation in *Drosophila*. *Evolution*, **43**, 362–381.

Coyne, J.A. & Orr, H.A. (2004) *Speciation*. Sinauer Associates, Sunderland, MA, USA.

Currat, M. & Excoffier, L. (2005) The effect of the Neolithic expansion on European molecular diversity. *Proceedings of the Royal Society of London B: Biological Sciences*, **272**, 679–688.

David, H.A. (1988) *The Method of Paired Comparisons*. C. Griffin, London, UK.

Dijkstra, P.D. & Groothuis, T.G.G. (2011) Male-male competition as a force in evolutionary diversification: evidence in haplochromine cichlid fish. *International Journal of Evolutionary Biology*, **2011**, 689254.

Fisher, R.A. (1932) *Statistical Methods for Research Workers*. Oliver & Boyd, London, UK.

Gartrell, B.D., Girling, J.E., Edwards, A. & Jones, S.M. (2002) Comparison of noninvasive methods for the evaluation of female reproductive condition in a large viviparous lizard, *Tiliqua nigrolutea*. *Zoo Biology*, **21**, 253–268.

Gassert, F., Schulte, U., Husemann, M., Ulrich, W., Rödder, D., Hochkirch, A. *et al.* (2013) From southern refugia to the northern range margin: genetic population structure of the common wall lizard, *Podarcis muralis*. *Journal of Biogeography*, **40**, 1475–1489.

Giovannotti, M., Nisi-Cerioni, P. & Caputo, V. (2010) Mitochondrial DNA sequence analysis reveals multiple Pleistocene glacial refugia for *Podarcis muralis* (Laurenti, 1768) in the Italian Peninsula. *Italian Journal of Zoology*, **77**, 277–288.

Hartman, P., Wetzel, D., Crowley, P. & Westneat, D. (2012) The impact of extra-pair mating behavior on hybridization and genetic introgression. *Theoretical Ecology*, **5**, 219–229.

Heathcote, R.P., Dawson, D. & Uller, T. (2015) Characterisation of nine European wall lizard (*Podarcis muralis*) microsatellite loci of utility across sub-species. *Conservation Genetics Resources*, **7**, 85–87.

Heathcote, R.J.P., Bell, E., d'Ettorre, P., While, G.M. & Uller, T. (2014) The scent of sun worship: basking experience alters scent mark composition in male lizards. *Behavioral Ecology and Sociobiology*, **68**, 861–870.

Heathcote, R.J.P., While, G.M., MacGregor, H.E.A., Sciberras, J., Leroy, C., D'Ettorre, P. *et al.* (2016) Male behaviour drives assortative reproduction during the initial stage of secondary contact. *Journal of Evolutionary Biology*, **29**, 1003–1015.

Huyghe, K., Vanhooydonck, B., Herrel, A., Tadic, Z. & Van Damme, R. (2012) Female lizards ignore the sweet scent of success: male characteristics implicated in female mate preference. *Zoology*, **115**, 217–222.

Jones, A.G. (2009) On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. *Evolution*, **63**, 1673–1684.

Johnson, B.B., White, T.A., Phillips, C.A. & Zamudio, K.R. (2015) Asymmetric introgression in a spotted salamander hybrid zone. *Journal of Heredity*, **106**, 608–617.

Kenward, R.E., Walls, S.S., South, A.B. & Casey, N. (2008) *Ranges8: For the Analysis of Tracking and Location Data*. Anatrack Ltd, Wareham, UK.

Kie, J. (2013) A rule-based *ad hoc* method for selecting a bandwidth in kernel home-range analyses. *Animal Biotelemetry*, **1**, 1–12.

Kingsolver, J.G. & Pfennig, D.W. (2007) Patterns and power of phenotypic selection in nature. *BioScience*, **57**, 561–572.

Lande, R. & Arnold, S. (1983) The measurement of selection on correlated characters. *Evolution*, **37**, 1210–1226.

MacGregor, H.E.A., While, G.M., Barratt, J., dePérez i Lanuza, G., Carazo, P., Michaelides, S. *et al.* (2016) Data from: Experimental contact zones reveal causes and targets of sexual selection in hybridizing lizards. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.4pq11.

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

Mendelson, T.C. & Shaw, K.L. (2005) Sexual behaviour: rapid speciation in an arthropod. *Nature*, **433**, 375–376.

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

Morrison, S.F., Keogh, J.S. & Scott, I.A. (2002) Molecular determination of paternity in a natural population of the multiply mating polygynous lizard *Eulamprus heatwolei*. *Molecular Ecology*, **11**, 535–545.

Noble, D.W.A., Wechmann, K., Keogh, J.S. & Whiting, M.J. (2013) Behavioral and morphological traits interact to promote the evolution of alternative reproductive tactics in a lizard. *The American Naturalist*, **182**, 726–742.

Oliveira, R.F., Taborsky, M. & Brockmann, H.J. (2008) *Alternative Reproductive Tactics - An Integrative Approach*. Cambridge University Press, Cambridge, UK.

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

Panhuis, T.M., Butlin, R., Zuk, M. & Tregenza, T. (2001) Sexual selection and speciation. *Trends in Ecology & Evolution*, **16**, 364–371.

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

Pearson, S.F. & Rohwer, S. (2000) Asymmetries in male aggression across an avian hybrid zone. *Behavioral Ecology*, **11**, 93–101.

Pemberton, J.M. (2010) Evolution of quantitative traits in the wild: mind the ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 2431–2438.

Pérez i de Lanuza, G., Carazo, P. & Font, E. (2014) Colours of quality: structural (but not pigment) coloration informs about male quality in a polychromatic lizard. *Animal Behaviour*, **90**, 73–81.

Pérez i de Lanuza, G., Font, E. & Monterde, J.L. (2013) Using visual modelling to study the evolution of lizard coloration: sexual selection drives the evolution of sexual dichromatism in lacertids. *Journal of Evolutionary Biology*, **26**, 1826–1835.

R Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Reichard, M., Bryja, J., Ondrackova, M., Davidova, M., Kaniewska, P. & Smith, C. (2005) Sexual selection for male dominance reduces opportunities for female mate choice in the European bitterling (*Rhodeus sericeus*). *Molecular Ecology*, **14**, 1533–1542.

Rosenfield, J.A. & Kodric-Brown, A. (2003) Sexual selection promotes hybridization between Pecos pupfish, *Cyprinodon pecosensis* and sheepshead minnow, C-variegatus. *Journal of Evolutionary Biology*, **16**, 595–606.

Rosenthal, G.G. (2013) Individual mating decisions and hybridization. *Journal of Evolutionary Biology*, **26**, 252–255.

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 Behavior*, **35**, 274–283.

Salvi, D., Harris, D., Kaliontzopoulou, A., Carretero, M. & Pinho, C. (2013) Persistence across Pleistocene ice ages in Mediterranean and extra-Mediterranean refugia: phylogeographic insights from the common wall lizard. *Bmc Evolutionary Biology*, **13**, 1–18.

Schulte, U. (2008) *Die Mauereidechse: erfolgreich im Schlepptau des Menschen*. Laurenti, Bielefeld, Germany.

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

Schulte, U., Hochkirch, A., Lötters, S., Rödder, D., Schweiger, S., Weimann, T. *et al.* (2012a) Cryptic niche conservatism among evolutionary lineages of an invasive lizard. *Global Ecology and Biogeography*, **21**, 198–211.

Shuster, S.M. (2009) Sexual selection and mating systems. *Proceedings of the National Academy of Sciences of the United states of America*, **106**, 10009–10016.

Singhal, S. & Moritz, C. (2013) Reproductive isolation between phylogeographic lineages scales with divergence. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20132246.

Spencer, W.D. & Barrett, R.H. (1984) An evaluation of the harmonic mean measure for defining carnivore activity areas. *Acta Zoologica Fennica*, **171**, 255–259.

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.

Stinchcombe, J.R., Agrawal, A.F., Hohenlohe, P.A., Arnold, S.J. & Blows, M.W. (2008) Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? *Evolution*, **62**, 2435–2440.

Symonds, M.E. & Moussalli, A. (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology*, **65**, 13–21.

Verzijden, M.N., ten Cate, C., Servedio, M.R., Kozak, G.M., Boughman, J.W. & Svensson, E.I. (2012) The impact of learning on sexual selection and speciation. *Trends in Ecology & Evolution*, **27**, 511–519.

Webster, M.S., Pruett-Jones, S., Westneat, D.F. & Arnold, S.J. (1995) Measuring the effects of pairing success, extra-pair copulations and mate quality on the opportunity for sexual selection. *Evolution*, **49**, 1147–1157.

While, G.M., Michaelides, S., Heathcote, R.J.P., MacGregor, H.E.A., Zajac, N., Beninde, J. *et al.* (2015) Sexual selection drives asymmetric introgression in wall lizards. *Ecology Letters*, **18**, 1366–1375.

Whitehead, H. (2009) SOCPROG programs: analysing animal social structures. *Behavioral Ecology and Sociobiology*, **63**, 765–778.

Willis, P.M., Ryan, M.J. & Rosenthal, G.G. (2011) Encounter rates with conspecific males influence female mate choice in a naturally hybridizing fish. *Behavioral Ecology*, **22**, 1234–1240.

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

Worton, B.J. (1989) Kernel methods for estimating the utilization distribution in home-range studies. *Ecology*, **70**, 164–168.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Expanded materials and methods.

Fig. S1. Photograph of an enclosure used to house lizards during the experiment.

Table S1. Mean trait values presented by lineage and sex.

Table S2. Ethogram used during behavioural observations to collect data on male–male and male–female interactions within the experimental enclosures.

Table S3. Details on the six microsatellites used for the analysis of offspring paternity.

Table S4. Estimated home range areas by lineage and sex.

Table S5. Factor loadings for PC1_BodySize.

Table S6. Candidate models from multiple regression analyses to identify male traits associated with relative fertilization success.

Table S7. Variance inflation factors (VIFs) from multiple regression analyses to identify male traits associated with relative fertilization success.

Table S8. Results from tests for asymmetries between the lineages in home range area and male–female overlap in the enclosures.

Table S9. Pearson's correlation coefficients between male traits presented by lineage.

Tables S10–S12. Complete model selection tables from multiple regression analyses to identify male traits associated with relative fertilization success.

Table S13. Results from partitioning of the (co)variance in male reproductive success into within-lineage and between-lineage components.

Table S14. Standardized linear (β_i) and quadratic (γ_{ii}) regression coefficients from single-trait models with relative fertilization success within-lineage or between-lineage as the response variable.