Male behaviour drives assortative reproduction during the initial stage of secondary contact

R. J. P. HEATHCOTE*[1], G. M. WHILE*†, H. E. A. MACGREGOR*†, J. SCIBERRAS*,
C. LEROY‡, P. D'ETTORRE‡ & T. ULLER*§

*Department of Zoology, Edward Grey Institute, University of Oxford, Oxford, UK
†School of Biological Sciences, University of Tasmania, Sandy Bay, Tas., Australia
‡Laboratory of Experimental and Comparative Ethology, Sorbonne Paris Cité, University of Paris 13, Villetaneuse, France
§Department of Biology, Lund University, Lund, Sweden

Abstract

Phenotypic divergence in allopatry can facilitate speciation by reducing the likelihood that individuals of different lineages hybridize during secondary contact. However, few studies have established the causes of reproductive isolation in the crucial early stages of secondary contact. Here, we establish behavioural causes of assortative reproduction between two phenotypically divergent lineages of the European wall lizard (*Podarcis muralis*), which have recently come into secondary contact. Parentage was highly assortative in experimental contact zones. However, despite pronounced divergence in male phenotypes, including chemical and visual sexual signals, there was no evidence that females discriminated between males of the two lineages in staged interactions or under naturalistic free-ranging conditions. Instead, assortative reproduction was driven by male mate preferences and, to a lesser extent, male–male competition. The effects were more pronounced when the habitat structure promoted high lizard densities. These results emphasize that assortative reproduction can occur in the absence of female choice and that male behaviour may play an important role in limiting hybridization during the initial stages of secondary contact.

Introduction

A fundamental requirement for speciation is that populations reproduce assortatively. This may be initiated by morphological and behavioural divergence during geographical isolation, which limits interbreeding following secondary contact (Mayr, 1963). Precopulatory mechanisms such as heterospecific recognition and avoidance are clearly pivotal in maintaining reproductive barriers

Correspondence: R. J. P. Heathcote, Edward Grey Institute, Department of Zoology, University of Oxford, Oxford OX1 3PS, UK.
Tel.: +44 1392 724 694; fax: +44(0)1392724623;
e-mail: r.j.p.heathcote@gmail.com
and
T. Uller, Department of Biology, Lund University, Sölvegatan 37, 223 62 Lund, Sweden.
Tel.: +46 46 222 30 94; e-mail: tobias.uller@biol.lu.se
[1]Current address: Centre for Research in Animal Behaviour, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QG, UK.

in currently sympatric lineages. Indeed, many species that rarely hybridize in the wild will frequently do so in captivity (McCarthy, 2006), suggesting precopulatory mechanisms are important in maintaining species boundaries over and above post-copulatory factors such as hybrid sterility (Haldane, 1922; Hewitt *et al.*, 1987).

The majority of studies examining how behavioural mechanisms mediate gene flow are undertaken in established (ancient) hybrid zones (Coyne & Orr, 2004; Price, 2008; Abbott *et al.*, 2013). These studies have usually focussed on female choice, as the greater *per capita* investment in reproduction by females should result in their experiencing stronger selection against costly hybrid matings compared to males (Wirtz, 1999; Randler, 2002). This has led to the suggestion that divergence in traits involved in female mate choice due to sexual selection may play an integral role in speciation, facilitating heterospecific mating avoidance and reducing hybridization upon secondary contact (Lande, 1981; West-Eberhard, 1983; Panhuis *et al.*, 2001).

However, the mechanisms that mediate gene flow in established hybrid zones may not be representative of those important in the early stages of secondary contact. This is because these mechanisms are themselves likely to be the evolutionary consequences of costly hybrid matings (i.e. reinforcement) (Brodsky *et al.*, 1988; Wirtz, 1999; Tynkkynen *et al.*, 2004). Indeed, studies from invasive species suggest initial female discrimination is often weak due to a lack of historical selection and that secondary contact can result in rapid hybridization and loss of genetic integrity before reinforcement can occur (Echelle & Connor, 1989; Rhymer & Simberloff, 1996; Huxel, 1999; Muñoz-Fuentes *et al.*, 2007). Speciation may therefore be a much less frequent consequence of secondary contact than often believed, with homogenization of populations and lineage extinction being more common outcomes.

An important challenge in speciation research is therefore to identify the behavioural processes that can maintain barriers to gene flow before reinforcement has occurred. However, empirical studies documenting the phenotypic and ecological conditions that mediate hybridization during recent secondary contact are rare (see Grant & Grant (2009) for an exception). The role of male behaviour in these circumstances is in particular poorly understood, although there is a growing awareness of its importance in mediating hybridization between established species (e.g. Peterson *et al.*, 2005; Svensson *et al.*, 2007; Edward & Chapman, 2011). Due to their strong influence on intra- and interspecific interactions, environmental conditions during secondary contact may also play an important role in mediating hybridization (Doorn *et al.*, 1998; Boughman, 2001). For instance, hybrid matings are more likely when conspecific mates are rare (Hubbs, 1955; Pearson, 2000), implying that environmental conditions that increase local population density may contribute to the spatial and temporal variation in introgression often found in hybrid zones (such as in studies by Klingenberg *et al.*, 2000; Pearson, 2000; Bronson *et al.*, 2003). This factor may therefore be particularly important in reducing introgression during initial secondary contact, before strong conspecific mate preference has evolved.

In this paper, we experimentally tested how precopulatory behaviour promotes assortative mating between two phenotypically divergent lineages of the European wall lizard (*Podarcis muralis*). These lineages, one native to western Europe and the other native to central and western Italy, have recently come into secondary contact in several parts of Europe due to human introductions (Schulte *et al.*, 2012a; Michaelides *et al.*, 2013, 2015; While *et al.*, 2015a). Such contact zones created by human introductions provide excellent systems for studying secondary contact because the mechanisms and environmental factors mediating gene flow that are normally lost to history can be directly observed (Sax *et al.*, 2007; Blackburn *et al.*, 2009; Senn & Pemberton, 2009).

Our study had three main objectives. First, we aimed to establish the extent to which reproduction is assortative using replicated, semi-natural enclosures that mimicked secondary contact. Second, we tested whether assortativity depends on the spatial clustering of basking sites, an important resource for heliothermic (basking) lizard species that is likely to influence population density. Third, we used analyses of behavioural observations from our semi-natural enclosures combined with controlled behavioural trials to establish the relative contribution played by both male *and* female precopulatory behaviours (including those influenced by olfactory sexual signalling) in driving reproductive assortativity upon secondary contact.

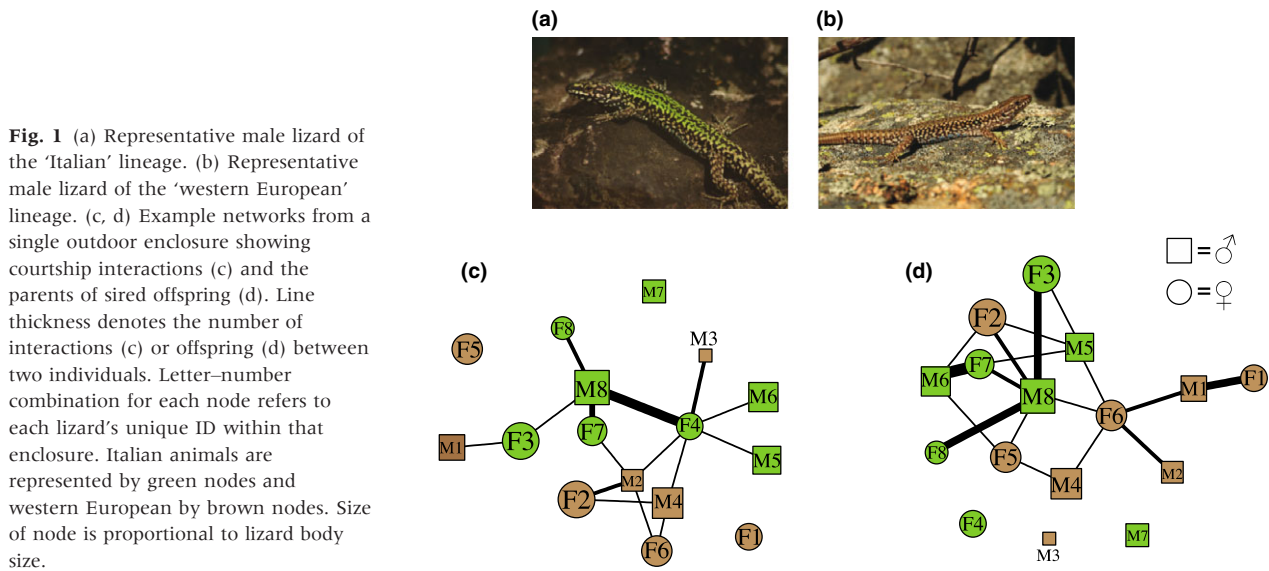## Materials and methods

Study species

The European wall lizard (*Podarcis muralis*) is a small (up to 75 mm snout-to-vent length (SVL)) diurnal lacertid native throughout western and southern Europe. Over the last 30–150 years, multiple non-native populations have become established in Germany, North America and the UK (Schulte *et al.*, 2012a; Michaelides *et al.*, 2013). Several of the introduced populations in Germany and the UK are composed of two main lineages (which we refer to as 'western European' and 'Italian') that separated around two million years ago (Schulte *et al.*, 2012b; Michaelides *et al.*, 2013, 2015).

All animals used in this study were from non-native UK populations (detailed below; Table S1). The animals we refer to as the western European lineage have haplotypes from the western or eastern France subclades, and the Italian lineage all have haplotypes from the Tuscan or Venetian clade (as per Schulte *et al.*, 2012a) and originate from the Tuscany or Bologna–Modena regions. The lineages differ conspicuously in dorsolateral coloration (brown in western Europe, green in Italy; Fig. 1) and the Italian lineage exhibits exaggeration of characters previously demonstrated to be under sexual selection in lacertid lizards and *Podarcis*, including relative head size, bite force, coloration and body mass (Bohme, 1986; Braña, 1996; While *et al.*, 2015a; see below).

Phenotypic differences between lineages

Morphometrics
All lizards that were used in the experiments described below had their SVL measured to the nearest 1 mm, their mass measured to the closest 0.01 g, and the width and length of the head measured to the nearest 0.1 mm. To determine how the lineages and sexes differed in body and head size, we ran two separate linear models, firstly with the first principal component

Fig. 1 (a) Representative male lizard of the 'Italian' lineage. (b) Representative male lizard of the 'western European' lineage. (c, d) Example networks from a single outdoor enclosure showing courtship interactions (c) and the parents of sired offspring (d). Line thickness denotes the number of interactions (c) or offspring (d) between two individuals. Letter–number combination for each node refers to each lizard's unique ID within that enclosure. Italian animals are represented by green nodes and western European by brown nodes. Size of node is proportional to lizard body size.

(PC) between head length and head width ('head size' from hereon) and secondly with the first PC between SVL and mass ('body size' from hereon; see Table S2 for details on the PCs) as response variables with lineage, sex and their interaction as predictors. In the models for head size, SVL was also included.

Chemical composition of male scent marks
Femoral secretions are used in scent marking and argued to play a major role in mate choice in lacertid lizards (Martín & López, 2014a,b). To determine whether the chemical composition of femoral secretions differed between the two wall lizard lineages, we caught 90 lizards (30 males and 15 female lizards of each lineage) from seven introduced populations of either Italian or western European origin (see supplementary information). All lizards were captured by noosing and transferred the same day to the facilities in Oxford, where they were kept individually in cages (800 × 400 × 500 mm (length × width × height)). All cages contained a basking spot, two hides made from bricks and tiles, and sand as substrate. Lighting and heat was provided by a 60W spot bulb placed over a brick on one end of the cage to provide a surface thermal gradient that ranged from approximately 45 °C to 22 °C, an EXO-TERRA™ 10.0 UVB fluorescent tube and overhead fluorescent room lighting. All lizards were provided with food (live crickets and mealworms) and water *ad libitum*.

Femoral pore secretions were collected from the males within 2 days of capture using sterilized forceps and then stored in sterile glass containers at −20 °C until chemical analysis (see Heathcote *et al.* (2014) for details). The lipophilic compounds in secretions were analysed by gas chromatography mass spectrometry (GC-MS) using an Agilent Technologies 7890A gas chromatograph coupled with an Agilent 5975 mass spectrometer, after first placing secretions in 150 μL of pentane. The GC peak areas were integrated using the Agilent Chemstation software, and the relative compound levels were calculated and then normalized with Aitchison's formula (Aitchison, 1986; Dietemann *et al.*, 2003). We identified compounds by initially comparing them to the mass spectral library in NIST 2008 and confirming these by comparison to pure synthetic compounds. We report identified and unidentified compounds by their retention time and characteristic ion signature.

We characterized 28 different lipophilic compounds, of which 15 were shared by all individuals (see Table S3 for details on chemical compounds). We performed a principal component analysis on the normalized peak area from the 15 shared compounds. The first seven PCs, which together explained 87% of total chemical variation, included the main compounds previously shown to be important in lizard olfactory communication (Mason & Parker, 2010; see Table S4 for eigenvector loadings). These seven PCs were then used to determine the overall difference in chemical signature between the two lineages of males by running a MANOVA with lineage and population as the two explanatory variables. After obtaining a significant effect in the MANOVAs, we carried out further 'Protected ANOVAs' on each principal component as a response variable, with the same predictors as the preceding MANOVA.

Behavioural discrimination and mate choice

Female olfactory discrimination
We next used the same 60 males and 30 females detailed above in a female discrimination and choice experiment. Females were captured while gravid with their first

clutch of the season, which they laid in the laboratory. Following oviposition, each female underwent three different behavioural trials with the same pair of size-matched (to within 3 mm) males (one from each lineage) to determine (i) whether females could discriminate between the different lineages, (ii) whether females preferred to associate and settle within the territories of same-lineage males in outdoor enclosures and (iii) whether courtship and mating were affected by the different male–female lineage combinations. These trials began three to seven days after a female laid her first clutch of eggs, which corresponds to the receptive period under laboratory conditions (R.J.P. Heathcote, G.M. While & T. Uller, unpublished data).

Tongue-flicking trials. Tongue flicking has been extensively used as a proxy for olfactory discrimination and mate choice in reptiles, particularly in lacertids (Cooper, 1994; Martín and López, 2014a). We therefore quantified a female's ability to discriminate between the scent marks of same- and different-lineage males by counting the number of 'tongue flicks' directed at scented cotton wool buds (Cooper, 1994; Barbosa *et al.*, 2006). Due to problems of functionally interpreting differential tongue-flicking rates (Heathcote *et al.*, 2014), we restrict our interpretation of tongue flicks to evidence for discrimination rather than mate choice. Scent was collected from males in a standardized manner by rubbing a sterile cotton bud along the length of the femoral pores of one leg. These cotton buds were then immediately presented to females. Each female underwent a separate tongue-flicking trial for each of the two males at the same time of day on two successive days. The presentation order of the two treatments was randomized for each female, and all trials were undertaken in the female's own cage, which was covered with one-way visual film to eliminate observer disturbances. Each male-scented bud was presented simultaneously 5 cm apart from a clean cotton bud, which acted as a control. Both buds were attached to a 30-cm-long wooden stick so they could be rested under the basking spot without disturbing the female, and thus removed any potential experimenter effects. The number of tongue flicks directed to each cotton bud was counted over a 30-min trial by an observer blind to the treatment.

Female association preferences. We then tested whether females were more likely to settle on sites scent marked by males of their own lineage. Male wall lizards are territorial, and a female's proximity to a male's scent-marked territory has been used as a standard means of testing mate choice (e.g. Martín and López, 2014a). Trials were undertaken two days after a female completed the tongue-flicking trials. All trials were run in large outdoor enclosures (~7 m × 7 m). Each enclosure had six basking sites, created from wooden pallets and breezeblocks. The basking sites in the outdoor enclosures were artificially scent-marked using the bricks, sand and hides obtained from the cage of the same pair of males whose scent was presented to a particular female during the tongue-flicking trials. The spacing between each basking site was approximately 80 cm, which represents the mean distance between male core territories in our semi-natural contact zone experiment. Each male's scent was randomly allocated to two basking sites, leaving the last two basking sites as unscented controls (which had sterile sand, bricks and slates added). The female was introduced into the enclosure, and her location was recorded every hour between her first emergence until she retired in the evening for four days, at which point she was captured and returned to the laboratory.

Staged mating experiment. Finally, after the two olfactory trials, the same female–male–male trio from the tongue-flicking and settlement trials was used in a staged mating experiment to determine lineage-based differences in courtship behaviour indicative of behavioural mate choice. All trials were carried out in clean cages identical in dimensions to those used to house the females. Each cage had a basking rock and shelter at both ends and a divider in the middle. The female and a randomly selected male from the trio were introduced into separate sides of the divider and allowed to acclimatize for 20 min. The divider was then lifted and the trial commenced. Trials ran for 60 min per male (or until a male mated), during which time we recorded the latency until courtship (seconds), whether the pair mated and the duration of mating (seconds). Once mated, the male was removed and the second male was immediately introduced. The order that males were introduced was randomized with respect to lineage. Cages were covered with one-way plastic to minimize disturbance to the animals during behavioural observations.

Male olfactory discrimination

Tongue-flicking trials. To determine whether males can discriminate between females based on olfactory cues, we captured an additional 90 lizards (30 females and 15 males from each lineage) from four populations (see supplementary information) in May 2012 and brought them into the laboratory. Scent was collected from females two days after oviposition of the first clutch by running a cotton bud down the length of a female's abdomen and tail base. Each male was presented with the scent of a female in a similar protocol to the female tongue-flicking trials. Because males (unlike females) rapidly habituated to the scented buds within a trial such that subsequent encounters with the cotton wool bud after the initial exposure only elicited very few tongue flicks, we only used the tongue flicks from the first encounter with the scented cotton buds

within each trial in our analysis. Because females do not have functional femoral pores and thus do not actively scent mark territories, we did not run the same association preference trials for males as detailed above for females.

Statistical analysis of behavioural discrimination

For both male and female tongue-flicking trials, individuals responded much more strongly towards the scented buds than the control buds (females: 14.9 ± 1.7 (mean $\pm$ SE) tongue flicks per scented cotton bud vs 3.7 ± 0.8 for the control; likelihood ratio test: $\chi^2 = 51.62$, $P < 0.001$; males: 35.0 ± 3.5 (mean $\pm$ SE) tongue flicks per scented cotton bud vs 8.9 ± 1.9 for the control; likelihood ratio test: $\chi^2 = 59.04$, $P < 0.001$). To test for differences in responses to same-lineage *vs.* different-lineage individuals, we therefore analysed the responses to the scented cotton buds only using generalized linear mixed models (GLMMs) with Poisson distributions and log-link functions using R statistical software (R Core Team, 2013). For both sexes, we included the total number of tongue flicks directed towards the scented cotton buds as a response variable, with male–female lineage combination (i.e. a factor with four levels), trial order (i.e. 1st vs 2nd), body size of the scented individual and an interaction between body size of the scented individual and lineage combination. The focal individual's ID was included as a random effect. Female locations at each pallet were pooled and analysed in two separate Poisson GLMMs with observations on a treated pallet as the response. The first included three levels of treatment (same lineage, different lineage, control), whereas the second omitted the control treatment to be able to include male body size as a covariate. Female ID was included as random effects in both models. For the staged mating trials, latency to court was log-transformed, and we ran separate mixed models for each of our response variables (latency to court (LMM), mating (yes/no) (binomial GLMM) and mating duration (LMM)). In these models, we included lineage combination and female body size as predictors.

In all analyses using Poisson and binomial responses distributions, we tested for, and if necessary, controlled for overdispersion in our data by including an observation-level random effect, allowing the scale parameter to be correctly modelled to validate a Poisson/binomial distribution (Elston *et al.*, 2001). The significance of factors in all mixed models was analysed using likelihood ratio tests on models with and without the factor of interest (Valdar *et al.*, 2006; Öckinger *et al.*, 2010).

Replicated contact zone experiment

Enclosure and resource treatment set up

We used ten large outdoor enclosures (~7 m $\times$ 7 m) to simulate an artificial secondary contact zone between lizards from the western European and Italian lineages.

Lizards populating these enclosures were caught in May 2010 from nine UK populations that, according to nuclear and mitochondrial genetic data, consist of pure western European or Italian lineages (see supplementary information for populations). Each enclosure housed eight males and eight females. All enclosures had an equal number of males of both origins. Three enclosures were stocked with an equal number of females from both origins but, due to limited sample size, five enclosures had five females of Italian and three females of western European origin and one enclosure was left with a single western European female and seven Italian females.

Within each enclosure, we constructed nine separate structures that made suitable basking resources, each made from wooden pallets (each one 1 m^2) and six breezeblocks, providing lizards with areas to forage, shelter and thermoregulate. To examine the extent to which these structures may influence wall lizard behaviour, we varied their spatial distribution to create our experimental treatment of resource distribution ('treatment' from hereon). In five enclosures, these resources were spaced equally apart in a 3×3 arrangement, each pallet approximately 80 cm apart ('dispersed treatment'), and in the other five enclosures, the 3×3 pallet arrangement was clumped together into the centre of the enclosures ('clumped treatment'). All individuals had a tissue sample taken (approx. 3 mm tail tip) for DNA analysis prior to release into the enclosures.

Lizards were marked with small (~7 mm diameter) yellow and white tags (made from Tesa® tape) on their dorsal surface, allowing them to be individually identified in the enclosure. Males were released into the enclosures on the 8th May 2010 five to seven days earlier than females to enable them to establish territories. Females were introduced to the enclosures following oviposition of their first clutch, with all females in a given enclosure introduced at the same time. This procedure made sure that all females completed an entire ovarian cycle in the enclosures (i.e. prereceptive, receptive, post-receptive) and, because sperm storage does not occur in this species (Pellitteri-Rosa *et al.*, 2012), only males from the enclosures could father any resulting offspring.

Behavioural data collection

Animals were observed daily for 26 days by three of the authors (RJPH, HEAM and JS). Each observation period for an enclosure lasted one hour before rotating to the next enclosure, and observations started when lizards first emerged in the morning and the last observation period finished after the last lizard retired to its shelter in the evening, providing an accumulated observation period of ~510 h. During observations, we recorded male and female identities in courtships and prolonged close male–female associations ('mate guarding', which allows males to monopolize mating access

to single females (Olsson, 1993; Galán & Price, 2000)), and male identities in agonistic encounters (win/lose; defined by when an individual retreated from another that was showing an agonistic display).

In addition to behavioural observations, we recorded the locations of all visible lizards every hour throughout their activity period each day. This location data allowed us to calculate the core home range size in m^2 for each lizard using Ranges v8 (Anatrack Ltd, Wareham, UK), with 'core home ranges' determined by calculating the 50% kernel cores for each lizard, using a least squares cross-validation smoothing parameter (Powell, 2000). The 50% kernel was chosen as it encompassed the majority of an animal's social interactions and delimited the onset of the plateau between the kernel percentage isopleth and the home range size (m^2) correlation, indicating the lizards were spending a high concentration of time in this area. Using the 50% kernel cores, we were able to determine the number of overlapping core home ranges for each individual lizard. We used this to determine whether the spatial distribution of basking sites influenced the density of lizards and whether this in turn influenced patterns of courtship and reproductive assortativity. We created four models, with males and females being analysed separately. The response variables analysed separately for each sex were core home range size in m^2 and total number of overlapping individuals. Core home range showed a left-biased skew and so was logged and used in LMMs, whereas the overlap data were analysed with Poisson GLMMs. For all four models, we included lineage, treatment and their interaction as predictors, and in the overlap models, we additionally included core home range size to control for this covariate. All models included enclosure as a random effect.

After the observational period, all lizards were captured and returned to the indoor facilities in Oxford. Females were housed individually in cages as detailed above. Eggs laid by females were incubated at 24 °C with a 5:1 vermiculite: water volume ratio. Upon hatching, tissue samples (tail tips) were taken of all juveniles to use in the genetic analysis.

Molecular analysis and paternity assignment
All adults and their resulting offspring from the enclosure experiment were genotyped at nine highly variable microsatellite loci that amplify across both subspecies as described in Heathcote *et al.* (2015). Paternity assignment was conducted in CERVUS v3.2 (Kalinowski *et al.*, 2007) using 10 000 simulations, with the mother's allele conformation being set as the known parent and all eight males she shared her enclosure with as the potential fathers. Six females nested in the enclosures before removal and these clutches were retrieved and assigned full parentage using CERVUS. A total of 19 females did not produce a clutch following

return to the laboratory. Paternity of the 296 juveniles from 61 clutches was confirmed at >99% confidence.

Social network and behavioural analyses
For each enclosure, we created 'home range overlap', 'courtship' and 'genetic' networks. Network 'edges' [i.e. interactions or associations between individuals (Croft *et al.*, 2008)] for the home range data were created between any two individuals that had overlapping core home ranges, in the courtship network between individuals observed courting and in the genetic networks between the parents of each offspring that was assigned parentage. We calculated the lineage-based assortativity for the three networks within each enclosure using the R statistical package 'assortnet', which allows the calculation of Newman's assortativity coefficients using weighted networks (R Core Team, 2013; Farine, 2014). To determine the statistical significance of assortativity, we compared the observed coefficient values within each network to a distribution of 10 000 randomly permuted matrices. To also test whether lineage-based assortativity differed between treatments, we calculated the *t*-statistic obtained by comparing the assortativity coefficient between the clumped and dispersed experimental treatments, and compared this to a random distribution of *t*-statistics generated from 10 000 permuted matrices.

Using Mantel tests, we tested whether the courtship and home range overlap networks correlated with the genetic networks for each enclosure, which would indicate that precopulatory male–female behaviours predict resulting paternity patterns [which may not be the case due to post-copulatory processes (Westneat, 1987; Olsson & Madsen, 1998)]. Mantel *t*-statistics for the correlation between networks for each enclosure were calculated and then compared to 10 000 permuted matrices to obtain our *P* value. To determine whether the enclosures differed in the degree to which the courtship networks predicted the genetic networks between the two treatments, we also compared the Mantel *t*-statistics obtained for each enclosure between the treatments using a *t*-test and compared this statistic to 10 000 permuted matrices to obtain our *P* value. We assessed the overall support for lineage-based assortativity and correlations between the different types of network by combining the results from each of the 10 enclosures using Fisher's omnibus test (Haccou & Meelis, 1992).

For the 58 males observed engaging in agonistic interactions in the enclosure experiment, we calculated their dominance using the David's score in SOCPROG analytical software (Whitehead, 2009). This was then used as a response variable in a LMM with male lineage, body size and their interaction as factors and enclosure as a random effect. Because dominance scores are nonindependent data, and thus violate the assumptions of a LMM, we calculated *P* values from

our models by comparing the model coefficients against those of randomized data sets obtained through a quadratic assignment procedure (where dominance scores were shuffled between individuals within an enclosure) with 10 000 permutations in R.

We determined the difference between the lineages in their reproductive success by constructing two Poisson GLMMs with total number of females courted and total number of offspring sired as separate response variables. In the courtship and sired offspring models, we included male lineage, treatment and their interaction as predictors. Enclosure was included as a random effect to control for the unbalanced number of females of different lineages in the different enclosures. Number of mate guardings were analysed in a Poisson GLMM with male ID and enclosure as random effects, and with male dominance score, the body size of the female he was observed guarding and lineage combination (a factor with four levels) as the predictors. To determine whether lineage-based differences in female traits might explain the reproductive patterns in the enclosure experiment, we analysed the number of courtships a female received in a Poisson GLMM, with lineage, treatment and body size as the predictors and enclosure as a random effect.

Predictors of hybridization
In addition to the network analyses, we also performed analyses of individual predictors of the number of hybrid offspring produced separately for each lineage and sex. For females, we only included animals that had at least one offspring assigned. We analysed animals of Italian and western European origin separately for two reasons. Firstly, because there were more Italian than western European clutches and the numbers of each lineage differed across enclosures, the strong assortativity would likely create spurious results for lineage-dependent hybridization in males. Secondly, the low incidence of hybridization for Italian females makes individual predictors of hybridization largely uninformative for Italian females and western European males.

Broadly speaking, in the absence of male and female preference for same-lineage mates, large dominant males with high reproductive success with same-lineage females should also produce more offspring with different-lineage females. On the contrary, if males prefer same-lineage females, smaller and less dominant males should produce more offspring with females from different lineages due to being excluded from their preferred mates. In male models, the number of hybrid offspring produced was therefore analysed in a Poisson GLMM, with the number of same-lineage offspring, dominance, core home range size, body size and treatment as fixed factors and enclosure as a random effect. For females, the number of hybrid offspring produced is limited by her clutch size. Female models were therefore analysed as a binomial GLMM (total number of

hybrid offspring over total number of offspring). All else being equal, larger females that receive more courtships (i.e. generally preferred females) should have a higher proportion of parentage with males of the dominant lineages. In addition, females with larger home ranges might hybridize with males from a less dominant lineage due to higher encounter rates, in particular with subdominant males. Our predictors for the female models were therefore body size, core home range size, number of courtships received and treatment, with enclosure as a random effect.

First-generation contact in free-ranging lizards

As an additional test of the degree of parentage assortativity, we analysed paternity from a population of lizards of mixed origin released as hatchlings in a disused quarry on the Dorset coast. The primary aim of the release was to investigate survival (see While *et al.*, 2015b), but here we analyse parentage data for eleven clutches of first-time breeders. Briefly, hatchlings of both origins were released close to existing lizard populations and recaptured in their second year after they had reached maturity. We recaptured 18 males (14 Italian, four western European) and 24 females (13 Italian, 11 western European), of which eleven females carried eggs at the time of capture. The females were brought to the animal facilities in Oxford where their eggs and offspring were processed as above. We analysed paternity by including all possible fathers as described above; all but one offspring could be assigned paternity with >99% confidence.

Results

Phenotypic differences between lineages

Morphometrics
Italian lizards were larger overall than western European lizards (lineage: $F_{1,157} = 6.23$, $P = 0.014$; sex: $F_{1,157} = 1.02$, $P = 0.313$; lineage×sex: $F_{1,156} = 0.04$, $P = 0.841$) with larger and more sexually dimorphic heads (lineage: $F_{1,156} = 31.23$, $P < 0.001$; sex: $F_{1,156} = 787.19$, $P < 0.001$; SVL: $F_{1,155} = 194.22$, $P < 0.001$; lineage×sex: $F_{1,155} = 6.55$, $P = 0.012$).

Chemical composition of male scent marks
Western European and Italian males differed significantly in the overall shared chemical composition of their femoral secretions (MANOVA; lineage: $F_{7,44} = 22.75$, $P < 0.001$; Population: $F_{35,240} = 1.05$, $P = 0.005$). Specifically, the two lineages differed significantly in values for PC1, which had strong positive loadings with cholesterol, hexadecanal, heptadecene and octodecanoic acid, and strong negative loadings with squalene, cholecalciferol (provitamin D) and campesterol (see Tables S3 and S4 for full chemical analysis results

and Table S5 for summary statistics). In addition, the femoral secretions from 28 of 29 Italian males contained high levels of α-tocopherol (vitamin E), comprising 11.62 ± 1.12% (mean ± SE) of their total chemical signature. In contrast, only 3 of 28 western European male secretions contained α-tocopherol, comprising 7.23 ± 1.93% (mean ± SE) of the chemical composition from these three individuals.

Behavioural discrimination and mate choice

Female olfactory discrimination

Tongue-flicking trials. Despite the pronounced differences in scent mark composition, females did not differ in the number of tongue flicks directed towards same- and different-lineage femoral secretions (Table S6).

Female association preferences. In the settlement trials, females preferred to settle on male-scented pallets over the controls ($\chi^2 = 13.30$, $P = 0.020$), but showed no preference for visiting a pallet scent marked by either same- or different-lineage males or for males of different body sizes (same vs different lineage: $\chi^2 = 0.17$, $P = 0.676$; male body size: $\chi^2 = 3.53$, $P = 0.061$).

Staged mating experiment. There was no influence of lineage or female body size on the latency to court (lineage combination: $\chi^2 = 6.53$, $P = 0.088$; female body size: $\chi^2 = 0.29$, $P = 0.593$). Lineage significantly predicted copulation likelihood, with Italian males being more likely than western European males to copulate with Italian females (lineage combination: $\chi^2 = 8.98$, $P = 0.029$; female body size: $\chi^2 < 0.01$, $P = 0.986$; see Table S7 for lineage-specific contrasts). For pairs that copulated, western European males copulated for longer with western European females than with Italian females (45.8±1.7 vs 36.4±2.2; mean±SE seconds), and Italian males copulated for longer with western European females than with Italian females (39.2±2.8 vs 33.5±2.5; mean±SE seconds) (lineage combination: $\chi^2 = 13.23$, $P = 0.004$; female body size: $\chi^2 = 0.21$, $P = 0.649$; see Table S8 for lineage-specific contrasts).

Male olfactory discrimination

In contrast to females, the number of tongue flicks by males directed towards female scent was significantly affected by the interaction between male–female lineage combination and female body size. Whereas Italian males tongue flicked less for larger females of both lineages, western European males showed the reverse response, and tongue flicked more overall when presented with scent from females of their own lineage (Table S6; Fig. S1).

Replicated contact zone experiment

Male core home range size showed a significant interaction between lineage and habitat structure, with males of western European origin having substantially larger home ranges in the dispersed habitat (Fig. S2). There were no significant predictors of female home range size. The experimental habitat manipulation caused differing densities of lizards, as evidenced from a greater home range overlap between lizards in the clumped habitat when controlling for core home range size (see Table S9 for all home range results).

Italian males were significantly dominant over western European males during territorial disputes (David's scores: Italian: 1.06 ± 0.41; western European: −1.26 ± 0.45 (mean ± SE) (lineage: $P = 0.006$; body size: $P = 0.255$; lineage×body size: $P = 0.086$). Of 58 interactions observed between Italian and western European males, we only observed five where the western European animal won (8.6%). Italian males also courted more females and sired a greater number of offspring than western European males, but there was no effect of experimental treatment or an interaction between lineage and treatment on either courtship or paternity (females courted: treatment: $\chi^2 = 0.20$, $P = 0.658$; lineage: $\chi^2 = 9.74$, $P = 0.002$; treatment×lineage: $\chi^2 = 0.28$, $P = 0.596$; number of offspring: treatment: $\chi^2 = 0.58$, $P = 0.445$; lineage: $\chi^2 = 8.33$, $P = 0.004$; treatment×lineage: $\chi^2 = 0.51$, $P = 0.477$). Larger females received more courtships than smaller females, but there was no statistically significant difference between lineages or treatment (treatment: $\chi^2 = 0.10$, $P = 0.757$; lineage: $\chi^2 = 2.07$, $P = 0.150$; body size: $\chi^2 = 4.91$, $P = 0.027$; Fig. S3). Mate guarding was predicted by high male dominance ($\chi^2 = 39.51$, $P < 0.001$), large female body size ($\chi^2 = 4.73$, $P = 0.029$), but not lineage combination ($\chi^2 = 0.54$, $P = 0.911$).

Social network analyses

Courtship networks were significantly assortative with respect to lineage ($\chi^2 = 45.96$, d.f. = 20, $P < 0.001$; Fig. 1). Furthermore, courtships were more strongly assortative towards the same lineage in the clumped compared to the dispersed enclosures ($P = 0.044$). Courtship networks correlated strongly with the genetic networks created from the paternity assignment ($\chi^2 = 55.66$, d.f. = 20, $P < 0.001$), showing that observed courtship behaviours were important in determining resulting paternity. As such, genetic networks were also highly assortative by lineage ($\chi^2 = 79.59$, d.f. = 20, $P < 0.001$; Fig. 1). Indeed, 83% (244 of 294) of overall offspring were sired by males from the same lineage. Assortativity in paternity networks did not differ between treatments (t = 0.69, $P = 0.290$). There was a borderline-significant effect of courtship observations being better predictors of paternity in the clumped treatment enclosures compared to the dispersed (t = 1.49;

$P = 0.058$). Networks based on home range overlap were not assortative by lineage ($\chi^2 = 16.41$, d.f. = 20, $P = 0.681$) and did not correlate with genetic networks ($\chi^2 = 15.78$, d.f. = 20, $P = 0.810$).

Predictors of hybridization

We were unable to identify individual male predictors of the number of hybrid offspring produced, including a lack of evidence that males that sired more offspring with same-lineage females sired more offspring with different-lineage females (Table 1). In the western European lineage, larger females (those that received more courtships) produced a higher number of hybrid offspring, whereas this was not the case in Italian females (Table 2).

First-generation contact in free-ranging lizards

The small sample size and low number of western European males preclude statistical inference, but parentage was highly assortative. Across the eleven clutches, all offspring in three of four clutches of western European females were sired by western European males (i.e. 73% of western European offspring were from assortative matings), whereas all but one offspring in one of seven clutches of Italian females were sired by an Italian male (i.e. 97% of Italian offspring were from assortative matings).

Discussion

The evolution of reproductive isolation is an important step in the speciation process. Although many authors suggest that phenotypic divergence during allopatry facilitates assortative mating through female choice (Wirtz, 1999; Price, 2008; Grant & Grant, 2009; Abbott et al., 2013), very few studies have attempted to identify the mechanisms underlying assortative mating in

Table 1 Summary statistics of Poisson GLMM testing for phenotypic predictors of the number of hybrid offspring produced by males from the enclosure experiment. Statistics for nonsignificant results are included at the point of their deletion from the model.

Model	Factor	χ^2	P
Italian male	Treatment	0.06	0.814
	Core home range	0.23	0.632
	Body size	0.18	0.668
	Conspecific offspring	0.45	0.503
	Dominance	2.62	0.106
Western European male	Dominance	0.19	0.872
	Conspecific offspring	0.20	0.656
	Body size	0.20	0.651
	Core home range	1.54	0.215
	Treatment	0.09	0.759

Table 2 Summary statistics of binomial GLMMs testing for phenotypic predictors of hybridization in females from the enclosure experiment. Significant factors are highlighted in bold. Statistics for nonsignificant results are included at the point of their deletion from the model.

Model	Factor	χ^2	P
Italian female	Body size	0.02	0.882
	Received courtships	0.03	0.857
	Core home range size	1.09	0.297
	Treatment	0.56	0.455
Western European female	Treatment	2.52	0.113
	Core home range size	1.69	0.193
	Received courtships	**6.68**	**0.010**
	Body size	**4.81**	**0.028**

the initial stages of secondary contact, before reinforcement has been able to occur.

The two lineages of wall lizard we discuss here have been isolated for ~2 myr (Gassert et al., 2013) and have subsequently diverged in their native range in many traits known to be important in male–male competition (body and head size, coloration, chemical signals, bite force), female choice (male body size, chemical signals) and male choice (female body size) in lizards (Tokarz, 1985; Olsson, 1993, 1994; Anderholm et al., 2004; Uller et al., 2010; Martín and López, 2014a,b; While et al., 2015a). The non-native populations used here retain these differences. As predicted from this period of isolation, paternity was highly assortative in our replicated, semi-natural contact zone populations, with 83% of offspring being produced by same-lineage parents, results that were replicated in a mixed origin free-ranging population. Below we discuss the relative contribution played by male and female behavioural traits in mediating these patterns.

Heterospecific mating avoidance is typically expected to be driven by females due to their greater *per capita* reproductive investment, which should lead to selection for more intense mate discrimination (Wirtz, 1999). Studies of natural hybrid zones have indeed shown that female choice is a major cause of reproductive isolation (e.g. Stein & Uy, 2006; Culumber et al., 2014). However, despite extensive phenotypic differences between the wall lizard lineages, including the chemical composition of their scent marks (the principal modality of female choice in lacertids, e.g. Martín & López, 2014b), we failed to find any evidence that female choice contributes to the maintenance of reproductive isolation. Females did not appear to discriminate between males based on olfactory cues; they did not prefer to settle in the territories of same-lineage males based on these olfactory cues; female home range overlap did not predict male paternity (as would be expected if females preferred to associate spatially with same-lineage males); and they were equally likely to accept males of

either lineage as mates in staged trials. Furthermore, males always initiated courtships in our replicated secondary contact zone experiment, the patterns of which strongly predicted resulting parentage. Taken together, this lack of evidence for female discrimination is consistent with the consensus view that precopulatory female choice is very limited in lizards (Olsson, 1993; Olsson & Madsen, 1995, 1998; Tokarz, 1995; Font *et al.*, 2012).

In the absence of female mate choice, barriers to hybridization should be weak as males are expected to show relatively low degrees of mate rejection. This should be particularly true in promiscuous species, such as the wall lizard, where males do not provide parental care and male *per capita* mating costs are assumed to be relatively low (Wirtz, 1999; Servedio, 2007). Despite this, levels of hybridization were low in both our semi-natural contact zones and first-generation free-ranging lizards. Our data from both naturalistic observations and laboratory trials suggest that this reproductive assortativity arises primarily from male preference for females of the same lineage. Male wall lizards of both lineages could distinguish between, and subsequently preferentially courted, same-lineage females, and male courtship patterns strongly predicted subsequent paternity. Although we cannot be certain of the specific female characters males use to assess potential mates, our data suggest some component of this is size-dependent since male courtship attention and olfactory discrimination, as well as female hybridization patterns, were predicted by female size. Because strong assortativity was also shown by the lizards that matured in our mixed origin natural population, such mate preferences are unlikely to have been learnt (Verzijden *et al.*, 2012), which otherwise could have contributed to the results from our enclosure experiments.

May other mechanisms have contributed to the assortative mating patterns we describe? Male Italian lizards are larger in size and exhibit greater exaggeration of intrasexually selected secondary characters. As a result, Italian males were competitively dominant over their western European counterparts during territorial disputes in the enclosures and hybridization primarily occurred between Italian males and western European females (see also While *et al.*, 2015a). Because large females were preferred by males of both lineages, this competitive asymmetry likely explains why Italian males were able to direct their courtship attention towards and monopolize matings with large females. This was particularly apparent in the mate guarding observations, where dominant (mostly Italian) individuals guarded the largest females. Such preference for larger and more fecund females is a common observation in lizards (Olsson, 1993). As Italian females are generally larger than western European females, this mechanism should contribute to reproductive assortativity even in the absence of other lineage-specific mate preferences that restrict mating

between individuals of different lineages. In support of this suggestion, larger western European females produced more hybrid offspring than smaller western European females, whereas there was no effect of female body size on incidence of hybridization in Italian females. As heterospecific mating avoidance may often only evolve in response to selection associated with costly hybrid matings (Brodsky *et al.*, 1988; Wirtz, 1999; Tynkkynen *et al.*, 2004), hybridization can be so rapid during initial secondary contact that lineages can become completely introgressed before reinforcement can occur (Echelle & Connor, 1989; Rhymer & Simberloff, 1996; Huxel, 1999; Muñoz-Fuentes *et al.*, 2007). Correlated evolution of male and female body size may therefore be an important factor that reduces gene flow in the early stages of secondary contact before strong conspecific mate choice has been able to evolve. For example, size-mediated hybridization has previously been shown in sticklebacks, where hybridization between two species that differ in overall body is most common between individuals in the overlapping size range (Nagel & Schluter, 1998; Conte & Schluter, 2013). However, in sticklebacks this hybridization is the consequence of females preferring similar-sized mates rather than through asymmetric male–male competition.

Our second aim was to determine whether small-scale differences in the clustering of suitable habitat influence behavioural processes that promote reproductive isolation. Such effects could partially explain the geographical variation in introgression frequently found in 'mosaic hybrid zones' (Barton & Hewitt, 1985; Sperling & Spence, 1991; Senn & Pemberton, 2009). In our experiment, courtships were more assortative when the habitat was highly clustered and home range overlap was subsequently high. This suggests that high population density facilitates the ability of males to choose their preferred females. Corroborating this, we found a borderline-significant effect of courtship networks being more predictive of parentage networks when resources were clumped. Considering our statistical power was low, this result suggests the possibility that precopulatory processes such as mate guarding and male monopolization may be more important in determining subsequent paternity when population densities are high. Despite this, we found no corresponding effect of habitat structure on assortativity in parentage nor did levels of hybridization differ between treatments. However, statistical power is limited in the network analyses and we may simply be unable to detect more modest effects. The contribution of post-copulatory isolation in this system and other secondary contact zones is worthy of further study but, nonetheless, the strong correlation between the courtship and genetic networks suggests that the degree of reproductive isolation documented here largely results from precopulatory processes.

In summary, and in contrast to most studies that emphasize the role of female choice in hybridization (e.g. Wirtz, 1999; Servedio, 2007), our results suggest that sexual selection acting on traits involved in male–male competition and male choice for female quantitative traits can promote assortative mating during initial secondary contact, as previously hypothesized for lizards (Barbosa *et al.*, 2006; Font *et al.*, 2012). Furthermore, the reproductive skew and monopolization of larger females by males of the dominant lineage in the enclosure experiment suggests that divergence in traits involved in male–male competition and correlated changes in female body size may contribute to assortative mating patterns during secondary contact. In this particular system, however, it is important to note that the degree of assortativity we report here is still insufficient to prevent introgression (While *et al.*, 2015a). Whether or not stronger degrees of mate preference have evolved in naturally occurring zones of secondary contact in this species warrants future investigation.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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.

Aitchison, J. 1986. *The Statistical Analysis of Compositional Data.* Chapman and Hall Ltd, London.

Anderholm, S., Olsson, M., Wapstra, E. & Ryberg, K. 2004. Fit and fat from enlarged badges: a field experiment on male sand lizards. *Proc. R. Soc. Lond. B* **271**: S142–S144.

Barbosa, D., Font, E., Desfilis, E. & Carretero, M.A. 2006. Chemically mediated species recognition in closely related *Podarcis* wall lizards. *J. Chem. Ecol.* **32**: 1587–1598.

Barton, N.H. & Hewitt, G.M. 1985. Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* **16**: 113–148.

Blackburn, T.M., Lockwood, J.L. & Cassey, P. 2009. *Avian Invasions: The Ecology and Evolution of Exotic Birds.* Oxford University Press, Oxford.

Bohme, W. 1986. Handbuch der Reptilien und Amphibien Europas (Band 2/II Echsen (Sauria) III, Aula-Verlag Wiesbaden.

Boughman, J.W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* **411**: 944–948.

Braña, F. 1996. Sexual dimorphism in Lacertid lizards: male head increase vs female abdomen increase? *Oikos* **75**: 511–523.

Brodsky, L.M., Ankney, C.D. & Dennis, D.G. 1988. The influence of male dominance on social interactions in black ducks and mallards. *Anim. Behav.* **36**: 1371–1378.

Bronson, C.L., Grubb, T.C. Jr, Sattler, G.D. & Braun, M.J. 2003. Mate preference: a possible causal mechanism for a moving hybrid zone. *Anim. Behav.* **65**: 489–500.

Conte, G.L. & Schluter, D. 2013. Experimental confirmation that body size determines mate preference via phenotype matching in a stickleback species pair. *Evolution* **67**: 1477–1484.

Cooper, W. 1994. Chemical discrimination by tongue-flicking in lizards: A review with hypotheses on its origin and its ecological and phylogenetic relationships. *J. Chem. Ecol.* **20**: 439–487.

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

Croft, D.P., James, R. & Krause, J. 2008. *Exploring Animal Social Networks.* Princeton University Press, Princeton.

Culumber, Z.W., Ochoa, O.M. & Rosenthal, G.G. 2014. Assortative mating and the maintenance of population structure in a natural hybrid zone. *Am. Nat.* **184**: 225–232.

Dietemann, V., Peeters, C., Liebig, J., Thivet, V. & Holldobler, B. 2003. Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. *Proc. Nat. Acad. Sci.* **100**: 10341–10346.

Doorn, G.S.V., Noest, A.J. & Hogeweg, P. 1998. Sympatric speciation and extinction driven by environment dependent sexual selection. *Proc. R. Soc. Lond. B* **265**: 1915–1919.

Echelle, A.A. & Connor, P.J. 1989. Rapid, geographically extensive genetic introgression after secondary contact between two pupfish species (*Cyprinodon*, Cyprinodontidae). *Evolution* **43**: 717–727.

Edward, D.A. & Chapman, T. 2011. The evolution and significance of male mate choice. *Trends Ecol. Evol.* **26**: 647–654.

Elston, D., Moss, R., Boulinier, T., Arrowsmith, C. & Lambin, X. 2001. Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology* **122**: 563–569.

Farine, D.R. 2014. Measuring phenotypic assortment in animal social networks: weighted associations are more robust than binary edges. *Anim. Behav.* **89**: 141–153.

Font, E., Barbosa, D., Sampedro, C. & Carazo, P. 2012. Social behavior, chemical communication, and adult neurogenesis:

Studies of scent mark function in *Podarcis* wall lizards. *Gen. Comp. Endocrinol.* **177**: 9–17.

Galán, P. & Price, A. 2000. Females that imitate males: dorsal coloration varies with reproductive stage in female *Podarcis bocagei* (Lacertidae). *Copeia* **2000**: 819–825.

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. J. Biogeogr.* **40**: 1475–1489.

Grant, P.R. & Grant, B.R. 2009. The secondary contact phase of allopatric speciation in Darwin's finches. *PNAS* **106**: 20141–20148.

Haccou, P. & Meelis, E. 1992. *Statistical Analysis of Behavioural Data: An Approach Based on Time-Structured Models*. Oxford University Press, Oxford.

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

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. *Behav. Ecol. Sociobiol.* **68**: 861–870.

Heathcote, R.J.P., Dawson, D.A. & Uller, T. 2015. Characterisation of nine European wall lizard (*Podarcis muralis*) microsatellite loci of utility across sub-species. *Conserv. Genet. Res.* **7**: 85–87.

Hewitt, G., Butlin, R. & East, T. 1987. Testicular dysfunction in hybrids between parapatric subspecies of the grasshopper *Chorthippus parallelus. Biol. J. Linnean. Soc.* **31**: 25–34.

Hubbs, C.L. 1955. Hybridization between fish species in nature. *Syst. Zool.* **4**: 1–20.

Huxel, G.R. 1999. Rapid displacement of native species by invasive species: effects of hybridization. *Biol. Cons.* **89**: 143–152.

Kalinowski, S.T., Taper, M.L. & Marshall, T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**: 1099–1106.

Klingenberg, C., Spence, J. & Mirth, C. 2000. Introgressive hybridization between two species of waterstriders (Hemiptera: Gerridae: *Limnoporus*): geographical structure and temporal change of a hybrid zone. *J. Evol. Biol.* **13**: 756–765.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *PNAS* **78**: 3721–3725.

Martín, J. & López, P. 2014a. Condition-dependent chemosignals in reproductive behavior of lizards. *Horm. Behav.* **68**: 14–24.

Martín, J. & López, P. 2014b. Pheromones and chemical communication in lizards. In: *Reproductive Biology and Phylogeny of Lizards and Tuatara* (J.L. Rheubert, D.S. Siegel & S.E. Trauth, eds), pp. 43–77. Taylor & Francis, Miami.

Mason, R. & Parker, M.R. 2010. Social behavior and pheromonal communication in reptiles. *J. Comp. Physiol. A* **196**: 729–749.

Mayr, E. 1963. *Animal Species and Evolution*. Harvard University Press, Massachusetts.

McCarthy, E.M. 2006. *Handbook of Avian Hybrids of the World*. Oxford University Press, Oxford.

Michaelides, S., While, G.M., Bell, C. & Uller, T. 2013. Human introductions create opportunities for intra-specific hybridization in an alien lizard. *Biol. Invasions* **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.

Muñoz-Fuentes, V., Vilà, C., Green, A.J., Negro, J.J. & Sorenson, M.D. 2007. Hybridization between white-headed ducks and introduced ruddy ducks in Spain. *Mol. Ecol.* **16**: 629–638.

Nagel, L. & Schluter, D. 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution* **52**: 209–218.

Öckinger, E., Schweiger, O., Crist, T.O., Debinski, D.M., Krauss, J., Kuussaari, M. *et al.* 2010. Life-history traits predict species responses to habitat area and isolation: a cross-continental synthesis. *Ecol. Lett.* **13**: 969–979.

Olsson, M. 1993. Male preference for large females and assortative mating for body size in the sand lizard (*Lacerta agilis*). *Behav. Ecol. Sociobiol.* **32**: 337–341.

Olsson, M. 1994. Nuptial coloration in the sand lizard, *Lacerta agilis*: an intra-sexually selected cue to fighting ability. *Anim. Behav.* **48**: 607–613.

Olsson, M. & Madsen, T. 1995a. 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* (T.R. Birkhead & A.P. Møller, eds), pp. 503–577. Academic Press, London.

Panhuis, T.M., Butlin, R., Zuk, M. & Tregenza, T. 2001. Sexual selection and speciation. *Trends Ecol. Evol.* **16**: 364–371.

Pearson, S.F. 2000. Behavioral asymmetries in a moving hybrid zone. *Behav. Ecol.* **11**: 84–92.

Pellitteri-Rosa, D., Sacchi, R., Pupin, F., Bellati, A., Cocca, W., Gentilli, A. *et al.* 2012. Testing the ability to store sperm: an experimental manipulation of mating opportunities in the common wall lizard, *Podarcis muralis. Acta Herpetol.* **7**: 111–118.

Peterson, M.A., Honchak, B.M., Locke, S.E., Beeman, T.E., Mendoza, J., Green, J. *et al.* 2005. Relative abundance and the species specific reinforcement of male mating preference in the *Chrysochus* (Coleoptera: Chrysomelidae) hybrid zone. *Evolution* **59**: 2639–2655.

Powell, R.A. 2000. Animal home ranges and territories and home range estimators. In: *Research Techniques in Animal Ecology: Controversies and Consequences* (L. Boitani & T.K. Fuller, eds), Columbia University Press, New York.

Price, T. 2008. *Speciation in Birds*. Roberts and Company, Greenwood Village.

R Core Team 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Randler, C. 2002. Avian hybridization, mixed pairing and female choice. *Anim. Behav.* **63**: 103–119.

Rhymer, J.M. & Simberloff, D. 1996. Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* **27**: 83–109.

Sax, D.F., Stachowicz, J.J., Brown, J.H., Bruno, J.F., Dawson, M.N., Gaines, S.D. *et al.* 2007. Ecological and evolutionary insights from species invasions. *Trends Ecol. Evol.* **22**: 465–471.

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. *Glob. 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.

Senn, H.V. & Pemberton, J.M. 2009. Variable extent of hybridization between invasive sika (*Cervus nippon*) and native red deer (*C. elaphus*) in a small geographical area. *Mol. Ecol.* **18**: 862–876.

Servedio, M.R. 2007. Male versus female mate choice: sexual selection and the evolution of species recognition via reinforcement. *Evolution* **61**: 2772–2789.

Sperling, F.A.H. & Spence, J.R. 1991. Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). *Evolution* **45**: 1370–1383.

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.

Svensson, E.I., Karlsson, K., Friberg, M. & Eroukhmanoff, F. 2007. Gender differences in species recognition and the evolution of asymetric sexual isolation. *Curr. Biol.* **17**: 1843–1947.

Tokarz, R.R. 1985. Body size as a factor determining dominance in staged agonistic encounters between male brown anoles (*Anolis sagrei*). *Anim. Behav.* **33**: 746–753.

Tokarz, R.R. 1995. Mate choice in lizards: a review. *Herpetol. Monogr.* **9**: 17–40.

Tynkkynen, K., Rantala, M. & Suhonen, J. 2004. Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. *J. Evol. Biol.* **17**: 759–767.

Uller, T., Stuart-Fox, D. & Olsson, M. 2010. Evolution of primary sexual characters in Reptiles. In: *The Evolution of Primary Sexual Characters in Animals* (J.L. Leonard & A. Cordoba-Aguilar, eds), pp. 425–452. Oxford University Press, Oxford.

Valdar, W., Solberg, L.C., Gauguier, D., Cookson, W.O., Rawlins, J.N.P., Mott, R. et al. 2006. Genetic and environmental effects on complex traits in mice. *Genetics* **174**: 275–281.

Verzijden, M.N., ten Cate, C., Sevedio, M.R., Kozak, G.M., Boughman, J.W. & Svensson, E.I. 2012. The impact of learning on sexual selection and speciation. *Trends Ecol. Evol.* **27**: 511–519.

West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Q. Rev. Biol.* **58**: 155–183.

Westneat, D.F. 1987. Extra-pair fertilizations in a predominantly monogamous bird: genetic evidence. *Anim. Behav.* **35**: 877–886.

While, G.M., Michaelides, S., Heathcote, R.J.P., MacGregor, H.E.A., Zajac, N., Beninde, J., Carazo, P., Perez I de Lanuza, G., Sacchi, R., Zuffi, M., Horvathova, T., Fresnillo, B., Schulte, U., Veith, M., Hochkirch, A. & Uller, T. 2015a. Sexual selection drives asymmetric introgression in wall lizards. *Ecol. Letts.* **18**: 1366–1375.

While, G.M., Williamson, J., Prescott, G., Horváthová, T., Fresnillo, B., Beeton, N.J. et al. 2015b. Adaptive responses to cool climate promotes persistence of a non-native lizard. *Proc. R. Soc. Lond. B* **282**: 20142638.

Whitehead, H. 2009. SOCPROG programs: analysing animal social structures. *Behav. Ecol. Sociobiol.* **63**: 765–778.

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

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 UK population origins of animals for each experiment [further details of these populations are found in Michaelides *et al.* (2013, 2015)].

Table S2 Details on morphometrics that comprised the principal components used in different parts of the study.

Table S3 Identified compounds from femoral pore secretions of male lizards, with their retention time and characteristic ion signature.

Table S4 Eigenvector loadings of the different chemical compounds for the seven main principal components.

Table S5 Summary of Protected ANOVAs conducted on individual principal components from the femoral pore extractions.

Table S6 Summary statistics of GLMMs for variables that predict the number of tongue flicks that male and female lizards direct towards scented-swabs.

Table S7 Separate contrasts from binomial GLMM for male-female lineage combinations on likelihood of copulation during the staged mating trials.

Table S8 Separate contrasts from LMM for male-female lineage combinations on duration of copulation during the staged mating trials.

Table S9 Summary statistics of mixed models for factors that predict home range size (in m^2) and the number of individuals overlapping in home range in the enclosure experiment for male and female lizards.

Figure S1 Number of tongue flicks from male lizards in response to scent from female lizards based on the interaction between lineage combination and female body size in a Poisson GLMM.

Figure S2 Core home range size (in m^2) for male French and Italian lizards in clumped and dispersed basking treatments.

Figure S3 Relationship between female body size and the number of courtships received from males in the replicated contact zone experiment.