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Optimal level of inbreeding in the common lizard

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Mate choice with regard to genetic similarity has been rarely considered as a dynamic process. We examined this possibility in breeding populations of the common lizard (*Lacerta vivipara*) kept for several years in semi-natural conditions. We investigated whether they displayed a pattern of mate choice according to the genetic similarity and whether it was context-dependent. Mate choice depended on genetic similarity with the partner and also on age and condition. There was no systematic avoidance of inbreeding. Females of intermediate ages, more monogamous, did not mate with genetically similar partners, whereas younger and older females, more polyandrous, did but highest clutch proportions were associated with intermediate values of pair-relatedness. These results indicate dynamic mate choice, suggesting that individuals of different phenotypes select their partners in different ways according to their genetic similarity. We consider our results in the light of diverse and apparently contradictory theories concerning genetic compatibility, and particularly, optimal inbreeding and inclusive fitness.

Keywords: *Lacerta vivipara*; mate choice; inbreeding; outbreeding; inclusive fitness; local conditions

1. INTRODUCTION

The evolution of mate choice with respect to genetic similarity with the partner has received considerable attention in the past 20 years because of its impact on dispersal evolution, fitness and conservation of small populations. Researchers have investigated the negative effects on fitness of both inbreeding (mating between two closely related individuals) and outbreeding (mating between two genetically distant partners). Owing to the deleterious effect of inbreeding (called inbreeding depression; Charlesworth & Charlesworth 1987; Keller & Waller 2002), individuals were until recently thought to avoid closely related partners (Tregenza & Wedell 2000) and even to seek-out genetically distant partners (outbreeding, Ober *et al.* 1997; Amos *et al.* 2001; Olsson *et al.* 2003). However, some exceptions to these generalities have been reported: inbreeding tolerance (Bateson 1982; Neff 2004) and selection of genetically similar partners (Cohen & Dearborn 2004) have both been demonstrated. In such cases, mating between two genetically distant partners could be disadvantageous (called outbreeding depression; Parker 1979), and should consequently be avoided. This reduction in fitness is mostly due to the break down of co-adapted gene complexes (Tregenza & Wedell 2000). To give a common theoretical framework to these apparently discordant results, it has been suggested that selection for inbred mating could depend on the costs and benefits of mating with a genetically close or distant partner. The optimal balance between costs and benefits will be achieved for a particular genetic distance between partners and will determine the optimal degree of inbreeding/outbreeding (Shields 1993); this is likely to be species specific. The existence of such optimal level of outbreeding has been demonstrated in bluegill sunfish (*Lepomis macrochirus*) populations (Neff 2004).

There is no evident reason why the balance between costs and benefits of mating with a genetically distant or close partner should not vary between populations or between individuals within a population; populations and individuals face different ecological and social situations to which they have to adapt. Indeed, in sexual selection, it has been recognized that males and females, when they differ in parental investment, are not expected to pay the same cost to inbreeding (Dawkins 1976) and this potentially leads to sexual conflict (Parker 1979; see review by Parker 2006). As outbreeding can also be disadvantageous, it is therefore possible that the optimal level of inbreeding/outbreeding is sex-dependent, such that males and females may display different choices according to mate genetic similarity. In polygynous systems, the cost of inbreeding should be higher for females and counter-strategies might have developed to decrease this cost, involving either kin recognition (Lehmann & Perrin 2003) or postcopulatory behaviours (Parker 2006). This sex asymmetry may be further enhanced by inclusive parent fitness, which favours selection for inbred mating (Parker 1979). Indeed, an individual mating with a relative will gain direct fitness through its offspring but also indirect fitness by improving its relative mating success. Although both sexes might benefit from inbred mating if the cost of inbreeding is not

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too great, however, an inbred mating will benefit the male more than the female even if the potential cost of missing an outbred mating is low (Parker 2006).

Kokko & Ots (2006) developed models for studying inbreeding tolerance with respect to opportunity costs, among other factors (e.g. animal life-history traits, parental investment). They show that inbreeding tolerance (and therefore mating with genetically close partner) should be substantially higher when mate choice is sequential rather than simultaneous, i.e. when the search cost is higher. Whenever individuals/populations show diverse mating strategies and mating opportunities, individual/population heterogeneity with respect to inbred mating is expected. Indeed, Neff (2004) found that the optimal level of outbreeding was dependent on the individual, but there have been few such studies so evidence is scarce.

We investigated the genetic distance between partners and its variation between individuals and populations. We choose the common lizard (*Lacerta vivipara*) as our model system, because this species potentially faces selection on mate choice in two opposite directions. Dispersal is relatively limited in this species and individuals tend to be sedentary; this enhances the opportunity of encountering genetically similar sexual partners within a population, which could select for inbreeding avoidance. Indeed, it has been shown in an ecologically closely related species that individuals avoided inbreeding (Olsson *et al.* 2003). However, females of the common lizard display different life-history strategies (Vercken *et al.* 2007) and the species can be found in habitats with different ecological characteristics (Lorenzon *et al.* 2001). This should select for mate preferences either within compatible life-history strategies or with the same set of co-adapted genes, two factors expected to favour inbred mating.

In this species, mate choosiness, and in particular the extent of females polyandry (Richard *et al.* 2005), changes with age and also depends on population characteristics, mainly the operational sex ratio (OSR; Fitze *et al.* 2005; Richard *et al.* 2005). As both the degree of polygynandry and mating opportunity change with age, there should be age-specific mate choice with respect to mate genetic similarity (prediction 1). Males are polygynous and do not provide any parental care. We therefore predict that males should be more tolerant to inbred mating than females (Dawkins (1976), prediction 2). Males emerge before females (by almost a month, Clobert *et al.* (1994)) and mate as soon as females emerge from hibernation. As not all females emerge at the same time (de Fraipont *et al.* 2000; Fitze *et al.* 2005), mating is more sequential than simultaneous in this species (see also the species description section 2a). Therefore, it is expected to have some inbreeding tolerance (prediction 3) in this species (Kokko & Ots 2006; Parker 2006). However, juvenile dispersal is partially driven by kin competition (Ronce *et al.* 1998; de Fraipont *et al.* 2000; Le Galliard *et al.* 2003), and close inbreeding avoidance is strongly suspected to explain the observed sex-dependent juvenile dispersal rate (Le Galliard *et al.* 2003). Accordingly, this close inbreeding avoidance, in combination with inbreeding tolerance (the third prediction), should result in there being an intermediate optimal level of inbreeding (prediction 4). Consequently, this system allows us to test various theoretical predictions concerning the role of mating system, sex asymmetry in parental investment and mating opportunities in mate choice with respect to genetic similarity (Kokko & Ots 2006; Parker 2006).

2. MATERIAL AND METHODS

(a) *The species*

The common lizard (*L. vivipara*) is a small ground-dwelling viviparous lizard (adult snout-vent length (SVL): 50–70 mm, SVL at hatching: 15–25 mm) inhabiting moist habitats across Eurasia. Populations can be structured into three distinct life-history stages: juveniles (year born); yearlings (1 year old); and mature adults.

Adult males emerge first from hibernation, in April, followed, a month later, by yearlings of both sexes (sexually immature individuals) and sexually mature females. As the males mate with the females as they emerge, male mating is mainly sequential. There is a strong male–male competition for access to the best females (Fitze *et al.* 2008) and female quality is age-dependent (Richard *et al.* 2005): intermediate age females are of better ‘quality’ than young and old females. Consequently, at same population sex ratio and density, depending on their age and male–male competition intensity, female mating can be either sequential (e.g. young and old females, low male–male competition) or simultaneous (e.g. medium age females, strong male–male competition).

In natural populations, survival of both males and females is age-dependent with an average adult survival of 40 per cent for males and 60 per cent for females per year, respectively (Ronce *et al.* 1998). Adult females reproduce once a year and the clutch size is between 1 and 12 eggs, with a mean of five, depending on body size (Boudjemadi *et al.* 1999). There is no parental care, hatchlings being autonomous at birth; there is also no apparent territoriality (Lecomte *et al.* 1994).

We selected the common lizard as our model system because of several of its characteristics. First, it is a promiscuous species with both monogamous and polygamous strategies coexisting (Laloi *et al.* 2004; Richard *et al.* 2005). Life-history traits are age-dependent (e.g. Ronce *et al.* 1998), as are mating strategies (Richard *et al.* 2005). Nevertheless, mate choice in this species is still poorly understood. For example, it is still unclear which is the selective sex and what criteria are used for mate selection, other than body size. As part of a long-term study on the common lizard we used individually marked (toe clipping) males and females of known age in semi-natural enclosures (Boudjemadi *et al.* 1999) to study mating patterns with regard to local conditions and genetic similarity.

(b) *Experimental design*

In June 1995, individuals were captured after the mating season (early June) in natural populations (non-genetically structured, M. Richard 2002, unpublished data) in the Massif Central (Mont Lozère, Lozère, 44°30′ N, 3°45′ E, altitude 1420 m) and were introduced into each of the four 100 m^2 unconnected enclosures in Foljuif Research Centre, near Paris (42°16′ N, 2°42′ E, altitude 200 m). The structure of each population is as follows: six postgravid females and their offspring (average of five offspring per female with an 11 : 9 male-to-female sex ratio); four adult males; and five yearlings of each sex. The transplanted population had similar age and sex structure as natural populations (Boudjemadi *et al.* 1999).

At introduction, all individuals were marked and tissue samples were collected. The number of reproducing individuals increased from 24 females and 16 males in 1995 to 27 (20 adults) females and 25 (20 adults) males in 1997, then to 36 (22 adults) females and 40 (29 adults) males in 1998. The number of reproducing adults was generally stable but the overall number of breeders increased, mainly because individuals started to reproduce younger, at the age of 1 as in lowland populations (Richard *et al.* 2005). This did not modify the general properties of the population (no selection); a reciprocal transplant experiment demonstrated that this change was the result of phenotypic plasticity (Sorci *et al.* 1996). Consequently, populations in the enclosures had similar properties (age structure, age of first reproduction, reproductive system, mean clutch size, etc.) to those of natural populations at the same latitude and elevation (Boudjemadi *et al.* 1999), in particular, as concerns the mean extent of polygynandry (Laloi *et al.* 2004).

In 1997 and 1998, all individuals were recaptured (six sessions of recapture a year, capture probability of 100%, see Richard *et al.* (2005) for further explanations). One month after mating, pregnant females were brought to the laboratory and kept there until parturition (Massot & Clobert 2000; Le Galliard *et al.* 2005). At birth, offspring were individually marked, measured and weighed and tissue samples were collected. The mothers and offspring were then returned to their enclosure of origin. All individuals were of known age unless older than 4 years, in which case they were classified as 5 years old. A total of 128 individuals (63 females and 65 males) were studied for the number of effective mating partners (i.e. at least one offspring produced). Offspring survival was defined both individually (offspring alive or not in June, the year following birth, i.e. at sexual maturity) and per clutch, as the ratio of offspring alive to all offspring in June. The juvenile survival rate is well in the range of what is found in nature (from 0.6 to 0.15 depending on the year and density; Chamaillé-Jammes *et al.* 2006). Enclosures have been also followed in 1996 but for technical reasons we have not been able to genotype all the juveniles. Therefore, we did not use the year 1996 in the statistical analyses.

(c) *Genotyping and paternity assessment*

Genomic DNA was extracted from ethanol-preserved samples using the Perfect gDNA Blood Mini Isolation Kit for animal blood (Eppendorf). Individuals were genotyped using six microsatellite markers (Laloi *et al.* 2004). Polymerase chain reaction (PCR) was carried out in 10 µl [15–50 ng DNA, 50–200 nm of each primer, 300 µm DNTPs, 1 µl 10× incubation buffer (50 mm KCl, 10 mm Tris–HCl, 1.5 mm $MgCl_2$, 0.1% TritonX-100, pH 9) and 0.25 U of Taq DNA Polymerase (Qbiogene)] in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems). Samples were then run on an ABI 310 automated sequencer (Applied Biosystems). Allelic size was determined using Genescan software v. 3.7 by reference to the Genescan Rox 400HD size standard.

Loci were tested for deviations from Hardy–Weinberg (HW) equilibrium, linkage disequilibrium using Arlequin v. 3.1 (Excoffier *et al.* 2005): there was no evidence of null alleles and the mean number of alleles per locus was 17.2 (s.d. = 1.94) within the introduced population in 1995. There was no deviation from HW or Linkage disequilibrium. We assessed paternity each year for each enclosure (and in the same time check for HW equilibrium and null alleles) using Cervus software, v. 3.0 (Kalinowski *et al.* 2007). The exclusionary power ranged between 0.980 and 0.999 according to enclosure and year (see appendix 1 in the electronic supplementary material for simulation parameters). To keep the genotyping error rate at the lowest level, mismatches between a parent and its offspring detected during the analyses have all been checked and corrected when it was not due to a non-typed locus. Fathers have been assigned with a confidence interval of 99 per cent, all individuals had a minimum of five loci typed and a maximum of one mismatch was allowed.

(d) *Genetic similarity measurements*

Genetic relatedness between all individuals was determined with Kingroup software (Konovalov *et al.* 2004), which uses multilocus genotypic data to evaluate relatedness in a population. We used the index ML, the new maximum-likelihood estimator based on the non-negativity of likelihoods (Konovalov & Heg 2008). The genetic similarity is expected to be 0.5 on average for full siblings and 0.25 for half siblings in a randomly mating population, which was the case in our population; for years 1995 and 1996, values are given for young of unrelated parents (considered unrelated as ‘between young and mother mean ML’ $=0.52$; s.d. $=0.05$, $n=40$): half sibs mean ML $=0.31$ (s.d. $=0.08$, $n=20$); full sibs mean ML $=0.56$ (s.d. $=0.10$, $n=20$). In order to test the performances of both our markers and the method of estimating relatedness, we used individuals with known pedigree (born after the introduction). All the estimates were consistent with the relatedness obtained from the pedigree.

(e) *Statistical analyses*

For the variables analysed, we used mixed log-linear models to test for factors significance (procedure GENMOD, SAS Institute). Some males and females were present both in 1997 and 1998 (14 females and 16 males). We therefore included an individual effect (repeated measures, Kenward–Roger method) as random factor with the procedure glimmix (SAS Institute). We used several within and between subjects variance structure (unstructured, compound symmetry, heterogeneous, heterogeneous compound symmetry and variance components) and compared them by their models AIC. The variance components structure was always selected and the model fit to the data was always good (in all cases, $\hat{c}$-inflation factor—less than 1.24).

Dependent variables tested (with the procedure glimmix) in adults were: relatedness of mated pair (i.e. pairs producing at least one juvenile, one value per pair, $n=120$), mean relatedness between a female and all of her mates (one value per female, $n=55$) and mean relatedness between a male and all his mates (one value per male, $n=65$). Relatedness was treated as Poisson response variable. Explanatory variables for adults were individual age and age^2 (for assessing any nonlinear relationship between the variable of interest and age), OSR in the enclosure of origin, number of mates, corpulence (residuals from the regression between body mass and SVL), and their interactions.

In addition, we examined reproductive success by analysing juvenile survival (treated as a binomial response variable, $n=295$). Parents are included as an individual effect (repeated measures, Kenward–Roger method) as random factor with the procedure glimmix (SAS Institute). Explanatory variables tested were the clutch size, age and age^2 of the parents, corpulence of the parents, number of partners, OSR and pair relatedness.

Model selection was backward, starting with a model with the two-order interactions, and dropping from the model all effects that were not significant ($p > 0.05$). The final model only contained significant terms, and, when two-way interaction terms were significant, we retained the main effects even if not significant by themselves (McCullagh & Nelder 1989). The final model chosen was based on largest log-likelihood χ^2 and minimum improvement χ^2. The use of AIC selection criteria led to the same results.

Four unconnected enclosures were used as independent experimental replicates. Owing to demographic stochasticity, the relative number of males and females or OSR (defined as the ratio of males to females aged 1 year and more) differed between replicates (mean OSR$=0.59\pm0.11$). As reproductive strategies depend on the number of potential partners available (Richard *et al.* 2005), the OSR was used as a descriptor of the enclosure and year effect. In 1997 enclosure three experienced a strong drop in population size and there was only one mature male left. As we wanted to test the effect of polyandry, we therefore did not use these data in the analyses.

3. RESULTS

(a) *Mating patterns at the population scale*

The theoretical value was estimated by only taking into account the actual mated individuals, so called potential sexual partners. The observed distribution of mated pairs relatedness ($n=120$, mean$=0.068$, s.d.$=0.177$) was not statistically different ($F_{1,3782}=0.35$, $p=0.55$) from the theoretical value (mean$=0.057$, s.d.$=0.177$) calculated by including all potential sexual partners (for each pair, per population and year). Mean relatedness of males ($n=65$) with their sexual partners (0.108, s.d.$=0.200$) was not statistically different ($F_{1,125}=1.64$, $p=0.20$) from the theoretical mean (0.072, s.d.$=0.097$) calculated by including all potential sexual partners (for each male, per population and year). Similarly, for females ($n=55$) the observed and the theoretical mean relatedness, respectively 0.073 (s.d.$=0.161$) and 0.050 (s.d.$=0.054$), were not significantly different ($F_{1,105}=0.94$, $p=0.33$). See appendices 2 and 3 in the electronic supplementary material for further details concerning relatedness distributions. Thus, mating patterns at the population scale showed no sign of partner avoidance (i.e. no inbreeding avoidance). However, random mating patterns at the population level can result from non-random individual mating (Bessa-Gomes *et al.* 2003). We therefore analysed the data at the individual level.

(b) *Individual variability in mating patterns*

Mean parents' relatedness was strongly dependent on the partners' age for both females and males (see table 1). The effect of female's age appears to be more complex: there were significant interactions with the OSR and with the degree of polyandry (table 1, figure 1*a*: $F_{15,38}=3.24$, $p=0.0016$). Because males and females behaved differently, they were analysed separately.

Mean relatedness of a female with all her partners appeared to be minimal for intermediates ages (2 and 3 years old, mean female relatedness$=0.016$, s.d.$=0.081$) and was maximal for extreme ages (1 and 4–5 years old: mean pair relatedness 0.137 (s.d.$=0.204$) and 0.065 (s.d.$=0.166$), respectively; see figure 1*b*). To better assess the effect of age, we distinguished three age-classes: young females (1 year old), females of intermediates ages (2 and 3 years old) and old females (4 and 5 (or more) years old). This distinction is based on an earlier study, which showed that female mating strategies differ between these three age classes (Richard *et al.* 2005). The main finding was that for females of intermediate ages, the mean relatedness with her partners was influenced by female corpulence (residuals of the regression between body weight and SVL, $F_{1,18}=5.14$, $p=0.0234$, figure 2; non-significant for the other age classes); the mean relatedness was highest for high female corpulence.

Table 1. Factors associated with female mean pair relatedness with all her partners (glimmix procedure). (We used mixed log-linear models with a backward selection. The final model only contains significant terms, and, when two-way interaction terms were significant, we retained the main effects even if not significant by themselves. Probabilities were obtained from the log-likelihood χ^2 (type 3 tests of the fixed effect). SAS presents the estimates relative to the previous factor, not the intercept.)

	estimate	s.e.	χ^2	$Pr\ (>\chi^2)$
intercept	5.1849	0.2041	645.04	<0.0001
OSR	−0.2022	0.3169	0.41	0.5224
female's age	−0.2746	0.0794	2.36	0.1243
female's age^2	0.0238	0.0098	0.02	0.8856
female's age × OSR	0.2467	0.1185	4.39	0.0361*
male's age	−0.0960	0.0224	18.35	<0.0001***
polyandrous status	−0.5462	0.1351	16.75	<0.0001***
female's age × polyandrous status	0.3218	0.1113	8.40	0.0037**
female's age^2 × polyandrous status	−0.0448	0.0192	5.45	0.0195*

The mean relatedness of males with their sexual partners was negatively associated with male age ($F_{1,61}=8.25$, $p=0.0056$) and age^2 ($F_{1,61}=4.44$, $p=0.0391$; figure 3). All other effects and analyses of relationships were non-significant (all $p>0.1$).

(c) *Juvenile survival*

Juvenile survival (table 2) was strongly influenced by the parent relatedness in interaction with maternal age and age^2. Paternal age (and interactions between paternal age and both OSR and the father polygynous status) also influenced juvenile survival indicating that the effect of inbreeding depression was dependent on parental age. Juvenile survival was also OSR-dependent but there was no significant interaction with parent relatedness (table 2). When considering only mothers, juvenile survival was significantly influenced by the interaction between female relatedness with her partners and her age ($F_{5,49}=9.06$, $p=0.004$) and age^2 ($F_{5,49}=8.14$, $P=0.006$; see figure 4). In fact, juvenile survival was highest when parent relatedness was between 0.1 and 0.3 for adult females (age>1 year); the maximal pair relatedness was 0.18 for females of intermediate age.

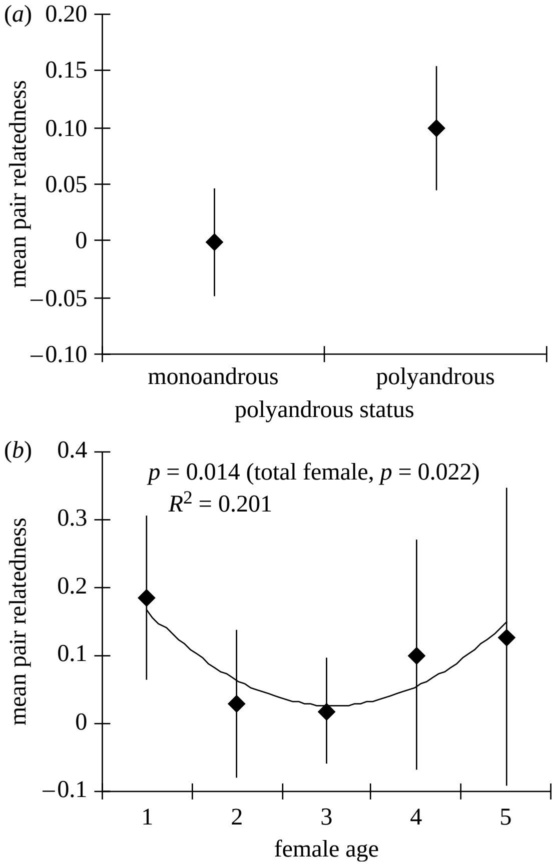

Figure 1. Influence of mating system and age on the female mean relatedness with their mates. Each point represents the mean for the category considered, and error bars indicate 95% confidence interval. (*a*) Mating strategy: mean pair relatedness for monoandrous ($N=16$) and polyandrous ($N=39$) females were -0.0003 (s.d. $=0.0972$) and 0.1000 (s.d. $=0.1748$), respectively, *t*-test, unequal variances ($F_{15,38}=3.24$, $p=0.0016$), $t=-2.79$, d.f. $=47.6$, $P(t)=0.0076$. (*b*) Female age in the subgroup of polyandrous females (age: $F_{1,36}=6.02$, $p=0.0185$; age^2: $F_{1,36}=4.98$, $p=0.0319$). All individuals were of known age unless older than 4 years, in which case they were classified as 5 years old. R^2 results from a regression between the data and the fit of the model (ProcGenmod, rel $=125.65-15.58\times$age$+2.50\times$age^2).

(d) *Pair relatedness and mating success*

We investigated whether the male contributions were equal for polyandrous females with regard to pair relatedness. Paternity in polyandrous broods was clearly non-equal: the male–female relatedness which was the closest to the optimal value (i.e. ML $=0.2$), was associated with the highest paternity (i.e. clutch proportion, 0.483 (s.d. $=0.233$) versus 0.284 (s.d. $=0.164$), $F_{1,101}=25.3$, $p<0.001$).

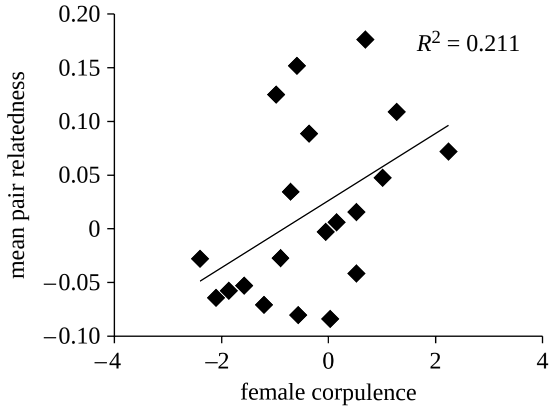

Figure 2. Female mean genetic relatedness with their mates according to female corpulence (residuals of the regression between body weight and SVL), for intermediate aged females (2 and 3 years old; $F_{1,18}=5.14$, $p=0.0234$). Each diamond represents one female. (Partial analysis with Proc-Genmod; fit equation: rel $=102.78+3.16\times$corpulence.)

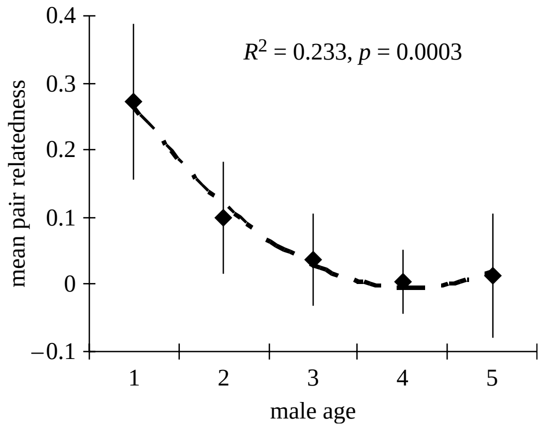

Figure 3. Males mean genetic relatedness with their mates varies with male's age and age^2 ($F_{1,61}=8.25$, $p=0.0056$ and $F_{1,61}=4.44$, $p=0.0391$, respectively). Each point represents the mean value for the category considered and error bars indicate 95% confidence interval (ProcGenmod, fit equation: rel $=147.52-24.12\times$age$+3.03\times$age^2).

4. DISCUSSION

We found no evidence for inbreeding avoidance at the population level (no departure from a random mating pattern). However, individuals displayed both age- and phenotype-dependent mean relatedness between partners. We also found that reproductive success was influenced by the genetic similarity between partners, but not in the same way for all age groups. Females of intermediate age with partners of intermediate genetic similarity ($0.1<\mathrm{ML}<0.3$) had higher reproductive success than other females. No such effect was found for males and the genetic relatedness with their partners. These findings further confirm that mate choice strategies cannot be fully described through analyses of population mating patterns (Bessa-Gomes *et al.* 2003).

(a) *Prediction 1: age-specific reproductive strategy and relatedness; females' active choice*

In the species studied, young (1 year) and old (4 and more years) females tend to be more polyandrous than intermediate aged females (Richard *et al.* 2005). Intermediate females may be of better quality and thus able to achieve their optimal strategy. Suboptimal females may mate with a larger number of males to increase their chances of obtaining at least one high quality sire (Richard *et al.* 2005). This pattern seems to correspond to an active choice by the female: when the OSR in a population increases, the proportion of monoandrous females is not affected, but the number of partners for the polyandrous females increases proportionally (Le Galliard *et al.* 2005). In addition, for polyandrous females, we found that the distribution of the genetic similarity of a female to her

Table 2. Factors influencing juvenile survival. (Final model (glimmix procedure). We used mixed log-linear models with a backward selection. The final model only contains significant terms, and, when two-way interaction terms were significant, we retained the main effects even if not significant by themselves. Probabilities were obtained from the log-likelihood χ^2 (type 3 tests of the fixed effect).)

	estimate	s.e.	χ^2	$Pr(>\chi^2)$
intercept	−7.6942	2.7915	7.60	0.0058
OSR	12.4988	5.1605	5.87	0.0154*
female's age	0.5353	0.5883	0.83	0.3629
female's age^2	−0.1077	0.0969	1.24	0.2660
pair relatedness	−9.6867	3.9002	6.17	0.0130*
clutch size	0.1463	0.0616	5.64	0.0176*
female's age × pair relatedness	8.1228	3.4126	5.67	0.0173*
female's age^2 × pair relatedness	−1.3769	0.5845	5.55	0.0185*
male's age	3.5101	1.2592	7.77	0.0053**
male's age × OSR	−6.8361	2.2708	9.06	0.0026**
polygynous status	−1.5005	0.8954	2.81	0.0938
male's age × polygynous status	0.7486	0.308	5.91	0.0151*

male partners was also bell-shaped: there was a higher mean relatedness between young and old females and their partners than between intermediate aged females and their partners (figure 1*b*). There was no such relationship for monoandrous females, and the variance in relatedness was very low (mean = 0.0003, s.d. = 0.0972, figure 1*a*). The female effect, introduced into our analyses as a random factor, did not interact significantly with any other explanatory variable. Therefore, selection cannot be an explanation of these patterns (Lindstrom & Kokko 2002).

There is another evidence for an active choice by the female: the positive relationship between female corpulence (residuals of the regression between body weight and SVL) and mean relatedness with her partners for intermediate aged females. There is no such relationship in the other age classes. In the common lizard, corpulence is usually considered as an indication of quality. Therefore, the best intermediate aged females are the most related to their partners in this age class (figure 2). This maximum value is also the optimum value, in terms of juvenile survival, for all adult females (age > 1, figure 4).

To our knowledge, this is the first time that a genetically based partner choice has been shown to be age and/or reproductive strategy-dependent. Only Dreiss *et al.* (2008) have reported that relatedness between partners depended on the condition of one member of the pair.

(b) *Prediction 2: differences in mating strategies and relatedness for males and females*

Males are polygynous and do not provide any parental care. We therefore predicted that males should be more tolerant to inbred mating than females. Indeed the males seemed to be less influenced by the mean relatedness to their partners. We found that the level of genetic relatedness with their mates depended only on age, decreasing as they aged (figure 3). Males of this species have a lower mean survival than females, and offspring survival is poorer for old males than for other males (Richard *et al.* 2005). Also, old males mate mainly with the oldest females, which are also of poor quality (Richard *et al.* 2005). If old males were choosing old females with low genetic similarity, it would be a strategy with a very low fitness pay-off. If there is indeed male mate choice in this species, it appears that high quality males (with the highest reproductive success in terms of mates and also of offspring survival, see also Richard *et al.* 2005) pair with females of intermediate relatedness, thereby avoiding close inbreeding. It is, however, difficult to disentangle active male choice and the consequences of age-dependent mate choice by females.

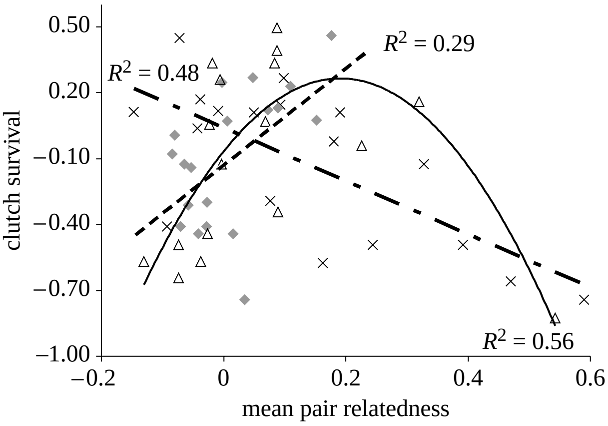

Figure 4. Clutch survival according to the female mean genetic relatedness with their partners. Each point corresponds to one female, and the three age classes are represented. The clutch survival is the residual of the survival by clutch size regression, corrected by year (statistical analyses reported in §3 were based on juveniles rather than clutches: the results are consistent). The optimal mean relatedness for adult females (age > 1 year) = 0.18. (Partial analysis with ProcGenmod; fit equations: age class 1, $\text{survival} = 0.039 + 1.211 \times \text{rel}$; age class 2–3, $\text{survival} = -0.116 + 2.141 \times \text{rel}$; age class 4–5, $\text{survival} = 0.067 + 3.475 \times \text{rel} - 9.101 \times \text{rel}^2$). Crosses, age 1; diamonds, age 2–3; triangles, age 4–5; long dashed-dashed line, fit (age 1); dashed line, fit (age 2–3); solid curve, fit (4–5).

(c) *Prediction 3: inclusive fitness and inbreeding tolerance*

The model of Kokko & Ots (2006) about inclusive fitness predicts that mating decisions may often differ between simultaneous and sequential scenarios: sequential choice would increase inbreeding tolerance (decrease in choosiness), whereas simultaneous choice applies where alternative mates are immediately available. In *L. vivipara*, males emerge first and mate with the females as they emerge subsequently. There is strong male–male competition for the best females, i.e. those of intermediate age (Fitze *et al.* 2008). The mate choice of the best females can be considered to be simultaneous and they are presumably choosier than males and than the other females: the best females are more often monoandrous. They are therefore expected, in this scenario, to be less tolerant toward inbreeding. By contrast, young and old females, less chosen by males, will have a sequential choice (for the same OSR). They should therefore be less choosy and have a higher tolerance to inbreeding. Indeed these females are more often polyandrous and they are more closely related to their mates than the intermediate aged females (mean relatedness 0.104 and 0.018 respectively; *t*-test, unequal variances ($F_{19,34} = 5.32$, $p = 0.0003$)

$t = -2.35$, d.f. $= 50.6$, $P(t) = 0.023$). Thus, the model of Kokko & Ots (2006) explains easily the 'bell shape' of the relationship between female's age and mean relatedness with mates. It is also consistent with the negative correlation between pairs' relatedness and OSR, as the choosiness should increase with mate encounter rate in this scenario.

(d) *Prediction 4: optimal inbreeding*

Although females rarely mated with very closely related partners (brothers), when such mating occurred offspring survival was strongly impaired (figure 4), indicating high inbreeding depression. However, for females mating with several males, the male–female relatedness closest to the optimal value (i.e. ML $= 0.2$) was associated with the highest paternity (i.e. clutch proportion, 0.483 versus 0.284, $F_{1,101} = 25.3$, $p < 0.001$). This seems to indicate post-copulatory selection. It is possible that the female either exhibits post-copulatory choice (Olsson *et al.* 1996; Pizzari *et al.* 2003) or that sperm function (Bretman *et al.* 2004) or competitive ability (Birkhead & Moller 1998) depends on relatedness. Also, juvenile survival depended strongly on female genetic relatedness with her sexual partners: juvenile survival was maximal for relatedness values approximately 0.2 and minimal at the extremes (figure 4). Consequently, it is possible to identify optimal inbreeding in this species (highest reproductive success in both clutch proportion and juvenile survival).

Most of the studies investigating correlations between offspring survival and parent mean relatedness only report negative effects of relatedness, although many only looked at the major histocompatibility complex, where loci are directly under selection (Edwards & Hedrick 1998). Very few studies have reported a bell-shape relationship with maximal fitness for an intermediate level of genetic relatedness. However, even if those studies have demonstrated an optimal level of relatedness, the causes and mechanisms generating this pattern remain largely unknown (Neff 2004).

Outbreeding depression could be generated by strong selection for local adaptation (Charlesworth 1994). Both the low dispersal capacity of the common lizard (Massot & Clobert 2000) and the level of landscape heterogeneity (humid habitat) are factors potentially selecting for local adaptation in this species. Indeed there are habitat effects on many traits (e.g. Chamaillé-Jammes *et al.* 2006) and evidence of local adaptation in this species (Clobert *et al.* 1994; Lorenzon *et al.* 2001); however, these local adaptations mainly arise through adaptive phenotypic plasticity (Lorenzon *et al.* 2001; but see Meylan *et al.* 2007). Reproductive strategies in this species are largely invariable—the level of polyandry is neither year nor population-dependent (Laloi *et al.* 2004; Le Galliard *et al.* 2005)—but we found age-dependent mating profiles to be also dependent on the population OSR (Richard *et al.* 2005; this study). Population OSR affected the shape of the relationship between offspring survival and the maternal mean relatedness with her partner. To what extent the bell-shape pattern results from local adaptation depends on the degree to which mate choice is condition-dependent with respect to the level of relatedness. Additional studies are required to elucidate this point.

5. CONCLUSION

Mating patterns in the common lizard were clearly influenced by the level of relatedness between partners, but not at the population level. Females mean relatedness with their partners was age-dependent and their reproductive success was highest for intermediate levels of relatedness. There was some optimal inbreeding in this species, with both an important inbreeding component, and strong outbreeding depression for partners that were more genetically distant than cousins. This trade off between inbreeding and outbreeding seems to operate through the action of plasticity, which is found in many other traits of this species: it can change with age for a given individual. The reasons for this type of optimal inbreeding pattern should, however, be more thoroughly investigated.

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