Research

CrossMark
click for updates

Authors for correspondence:
Luis M. San-Jose
e-mail: luis.sanjosegarcia@unil.ch
Patrick S. Fitze
e-mail: patrick.fitze@unil.ch

Royal Society Publishing

Cumulative frequency-dependent selective episodes allow for rapid morph cycles and rock-paper-scissors dynamics in species with overlapping generations

Luis M. San-Jose[1,2,3], Miguel Peñalver-Alcázar[2,3], Borja Milá[3], Virginia Gonzalez-Jimena[2,3] and Patrick S. Fitze[1,2,3,4]

[1]Department of Ecology and Evolutionary Biology, University of Lausanne, Lausanne 1015, Switzerland
[2]Instituto Pirenaico de Ecología, (IPE-CSIC), Jaca 22700, Spain
[3]Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid 28006, Spain
[4]Fundación Araid, Zaragoza 50018, Spain

Rock-paper-scissors (RPS) dynamics, which maintain genetic polymorphisms over time through negative frequency-dependent (FD) selection, can evolve in short-lived species with no generational overlap, where they produce rapid morph frequency cycles. However, most species have overlapping generations and thus, rapid RPS dynamics are thought to require stronger FD selection, the existence of which yet needs to be proved. Here, we experimentally demonstrate that two cumulative selective episodes, FD sexual selection reinforced by FD selection on offspring survival, generate sufficiently strong selection to generate rapid morph frequency cycles in the European common lizard *Zootoca vivipara*, a multi-annual species with major generational overlap. These findings show that the conditions required for the evolution of RPS games are fulfilled by almost all species exhibiting genetic polymorphisms and suggest that RPS games may be responsible for the maintenance of genetic diversity in a wide range of species.

1. Introduction

Rock-paper-scissors (RPS) games can give rise to evolutionary stable three-morph systems through negative frequency-dependent (FD) selection [1–3]. RPS dynamics result from fitness intransitivity whereby, each morph, when being the dominant morph, is vulnerable to invasion by another morph [1–3], resulting in periodically alternating morph predominance [4]. While the theoretical framework for the evolution of RPS dynamics and their ecological implications have been extensively studied (e.g. [3,5]), experimental evidence is scarce (e.g. [6]). Only a few biological examples have been described to date, including bacteria [7], insects [8] and reptiles [4]. However, negative FD selection has been described in other insect species [9], isopods [10] and plants [11], and remodelling of those systems suggested that negative FD selection might be the result of RPS dynamics [3]. All these species are short-lived and exhibit little or no generational overlap. This suggests that RPS dynamics might mediate the maintenance of polymorphisms in species covering most of the tree of life and thus that RPS dynamics might be very general mechanisms [3]. But on the other hand, it also suggests that the evolution of RPS dynamics might be restricted to narrow ecological conditions and thus to a small number of species, i.e. short-lived species, species with little or no generational overlap [3,12]. A few studies also suggest that RPS dynamics can govern interspecific interactions, for example competitive interactions among species, which suggests that they may affect a broader ecological scale, including community structures and the maintenance of biodiversity [13].

Theoretical models have shown that in short-lived species with little generational overlap, simple mechanisms can produce sufficient FD selection to

generate RPS games. For instance, in *Uta stansburiana* (the side-blotched lizard), a short-lived species with no reproductive overlap among different generations, negative FD selection on siring success of male colour morphs imposed by male–male competition is strong enough to induce rapid, 3–4 year morph frequency cycles [4,14,15]. By contrast, in most species stronger FD selection is required to generate rapid morph frequency cycles [16]. For example, in species with overlapping filial and parental generations, as occurs in the majority of the vertebrate species and in many vascular plants, individuals of the different generations mix and reproduce simultaneously. A bias in morph frequency produced in one generation, e.g. owing to FD selection during sexual selection, will thus only slightly change the population's morph frequency. FD selection of the magnitude observed in short-lived species would thus result in slow or no morph frequency cycles and only stronger FD selection can generate rapid cycles [17].

Rapid RPS-like morph cycles have been observed in at least one multi-annual species, *Zootoca vivipara* (the European common lizard) and a theoretical model suggests that stronger FD selection might be generated by multiple FD mechanisms acting in concert [16]. The European common lizard can live up to 13 years, and it has a pronounced age structure with significant generational overlap. Cohorts of maturing (1–2 years old) and mature males (3–4 years old) are distinguishable by body size [16,18–20], and overlap both temporally and spatially. Analogous to the orange, blue and yellow morphs of the side-blotched lizard, male common lizards exhibit distinct colour morphs that behave like a single locus with three alleles (orange, *o*, yellow, *y* and white, *w*; [16]) and can be classified using two colour scores [16], namely *o* and *y* score which account for the number of putative colour alleles (*o* score: $0 = ww, wy$, $1 = yo, wo$, $2 = oo$; *y* score: $0 = ww, oo$, wo, $1 = wy, yo$, $2 = yy$; figure 1a). Morphs can be distinguished by spectrophotometry and differential carotenoid deposition accounts for differences in white score [21]. *o* alleles positively and negatively affect endurance and survival, respectively [16]. *y* alleles have a dominant negative effect on body size [16], and *w* alleles confer higher survival and higher endurance, the latter through allometry. Adult male frequency is suggested to affect juvenile survival and high *w* frequency in the juvenile cohort increases the recruitment of other *w* juveniles, the latter suggesting the existence of cooperation as described in *U. stansburiana* [16,22]. In *Z. vivipara*, colour allele frequency cycles with a periodicity of 3–4 years at low and 6–8 years at high elevation have been observed in several different populations (figure 1b and fig. 5 in ref. [16]). A theoretical model showed that such cycles can be produced by strong FD selection resulting from the cumulative effects of two temporally distinct episodes of FD selection, namely FD sexual selection and FD survival selection on maturing juveniles [16]. Morph-specific survival of maturing juveniles is suggested to depend on adult morph frequency. More precisely, when *o* adults are common, *y* juveniles have higher, and *w* and *o* juveniles have lower survival. When *y* or *w* adults are common the survival of *y* juveniles is lower (fig. 5 and table 1a in ref. [16]). According to the model, FD survival selection on juveniles cannot produce strong enough selection to produce rapid morph frequency cycles, and thus additional mechanisms reinforcing the FD survival selection are required. In the model, such additional FD selection was provided by FD sexual selection. With other words, the model assumes that FD sexual selection on adult male morph (e.g. via

context-dependent female mate choice) and with respect to the progeny's survival advantage conferred by the father's morph provides enough FD selection to generate rapid morph frequency cycles. This indicates that sexual selection should be with respect to adult morph frequency prevailing during juvenile survival selection (the morph frequency of adult and maturing males, since the latter reach adulthood when the progeny will be exposed to survival selection in July/August), and not as in *Uta* with respect to the adult morph frequency present during the breeding season. The model further shows that rapid cycles only occur if each selective episode imposes a minimum selective pressure, namely a mating discrimination (mating success of sexually selected morphs relative to the success of the other morphs, *m*) above 1.3 and a survival advantage of the preferred morph's progeny above 1.08. Although the model explains the existence of rapid morph frequency cycles, no empirical evidence supports the existence and magnitude of such complex cumulative processes. Thus, it remains unclear whether RPS games represent a general mechanism that can potentially maintain polymorphisms in a wide range of species, or whether they are limited to a small number of species with particular life-history characteristics.

We experimentally tested the existence and the magnitude of the suggested cumulative processes using semi-natural populations of *Z. vivipara*. We established populations with similar adult colour morph frequency (intermediate between *w* and *o* adult male dominance), manipulated the frequency of the maturing cohort (i.e. the invading cohort), and thereby manipulated the direction of the morph frequency cycle (hereafter referred to as RPS phase). In half of the populations, we simulated advancement of the morph frequency cycle towards *o* by introducing maturing lizards with a morph frequency bias towards *o*, and in the remaining populations we introduced maturing lizards with a morph frequency bias towards *w* (figure 1c) [16]. We thus experimentally induced advancement and regression of the morph frequency cycle to test five predictions derived from theoretical models that are required to generate rapid morph frequency cycles in species with major generational overlap: (i) that adult colour morph is sexually selected; (ii) that sexual selection depends on male colour morph frequency, and more specifically (iii) that it depends on the adult morph frequency encountered by progeny at birth (i.e. on the frequency present when the invading cohorts will mature) and not on the adult frequency during sexual selection; (iv) that sexual selection is reinforced by a second temporally distinct selective episode, i.e. that the survival probability of the progeny of the most successful morph (during sexual selection) is higher than that of the progeny of other morphs; and (v) that the FD selection is of sufficient magnitude to produce rapid morph frequency cycles [16].

2. Material and methods

(a) Experimental populations

Semi-natural populations ($10 \times 10 \, m^2$) were enclosed by galvanized metal walls that were 1 m high and extended 1 m below the ground to prevent the escape of lizards and the entry of terrestrial predators. Each population contained planted natural grassland, two water ponds, logs and stone piles that provided lizards with hiding and basking sites. Each population was covered with a net to protect against avian predators and four irrigation sprinklers (one per corner) provided the high humidity
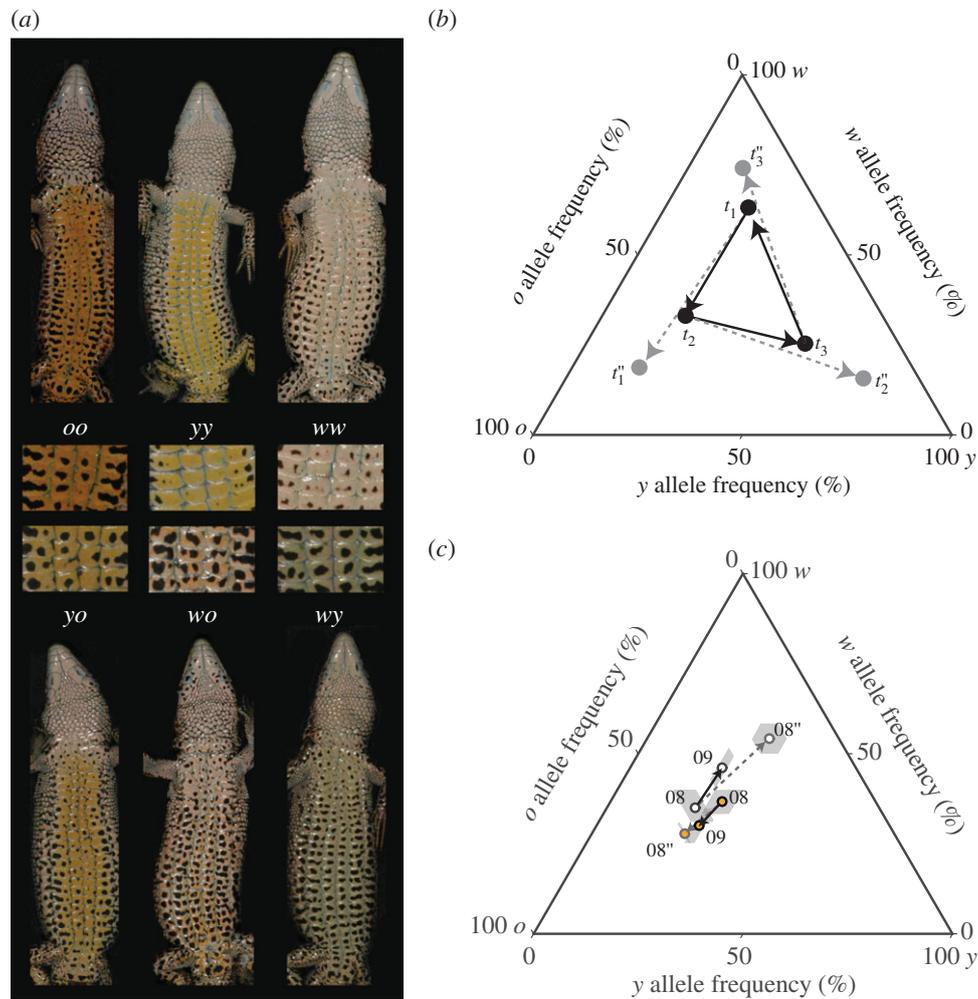
Figure 1. Colour morphs, morph frequency cycles and experimental design. (*a*) In the European common lizard, males exhibit colour morphs that behave like a single putative locus with three alleles (orange, *o*, yellow, *y* and white, *w*), producing six distinct phenotypes (homozygotes (*oo*, *yy* and *ww*) and heterozygotes (*yo*, *wy* and *wo*)). (*b*) Schematic showing 3-year RPS cycle of the common lizard. Morph frequencies of the mature cohort (black dots, t_x) exhibit $3-4$ year cycles. The maturing cohort's colour frequency is depicted with grey dots (t''_x). The morph frequency in the subsequent year (t_{x+1}) results from the newly mature cohort (t''_x) plus the surviving adults from the previous year (i.e. the surviving t_x adults). (*c*) Mature and maturing male cohorts were introduced into six experimental populations. Adult colour frequency (08) did not differ among RPS phases (see text). In three populations, we simulated an advancement of the morph frequency cycle towards *w* (white dots) by releasing maturing lizards (08″) with a *w* frequency bias and, in the remaining populations, advancement towards *o* by releasing maturing lizards with *o* bias (orange dots). Dashed arrows indicate the frequency difference among mature and maturing cohorts, and black arrows indicate the annual change in colour morph frequency of the mature cohorts from the time of their release (t_x; 08) until the following summer (t_{x+1}; 09, when the invading cohort completed maturation). Grey shaded areas depict the standard errors of the experimental colour morph frequencies.

conditions commonly found in high-density populations. In September 2008, we released into each population seven mature females (in Pyrenean populations females have whitish bellies and no yellow or orange belly coloration; [23]) and five to six mature males (more than 2 years old, snout to vent length (SVL) $\geq$ 51 mm; [16,24]), three maturing females and three to four maturing males (1 year old, $34 \geq$ SVL < 51 mm; [16,24]), and three female and three male juveniles (SVL $\leq$ 30 mm). The age structure and population density correspond to those observed in natural populations [25], and to natural high-density populations [26]. Moreover, similar population parameters have been used in other population experiments on this species [27].

The established adult colour morph frequency did not statistically differ between the RPS phases (*o*, *w*, *y* frequency $\pm$ s.e.: 36.7% $\pm$ 6.6, 36.7% $\pm$ 3.3, and 26.6% $\pm$ 3.3 for populations cycling towards *o* and 47.2% $\pm$ 2.7, 31.7% $\pm$ 4.4, 21.1% $\pm$ 6.7 for populations cycling towards *w*; $\chi_1^2 = 0.54$, $p = 0.46$) and the established colour morph frequency in the invading maturing cohort significantly differed between treatments (*o*, *w*, *y* frequency: 50.0% $\pm$ 0.0, 27.8% $\pm$ 2.8, and 22.2% $\pm$ 2.8 for populations cycling towards *o* and 16.7% $\pm$ 7.2, 54.2% $\pm$ 4.2,

29.2% $\pm$ 4.2; $\chi_1^2 = 6.10$, $p = 0.01$). We experimentally induced advancement and regression of the morph frequency cycle in order to disentangle the effects of the adult and adult + maturing male colour morph frequency on sexual selection. We used a single adult morph frequency distribution as baseline (i.e. a frequency intermediate between *w* and *o* biased) and three RPS phase replicates, which allowed us to measure the variability of the treatment effects. In an alternative design with all three intermediate adult morph frequencies (a frequency intermediate between *w* and *o*, intermediate between *y* and *o*, and intermediate between *w* and *y* biased), only one replicate per adult morph frequency and RPS phase combination would have been possible, and thus the variability could not have been estimated.

(b) Laboratory conditions

Released lizards hibernated from November 2008 until the beginning of the mating season in mid-March 2009. One month after the end of the mating season, all females were recaptured, individually housed in the laboratory until egg laying, and thereafter released in the outdoor enclosures. Individual terraria were

equipped with a shelter, a water pond and peat soil as substrate [28]. Water was provided ad libitum and females were fed every 3 days with alternating prey items (larvae of *Galleria mellonella*, *Acheta domestica* and *Lumbricus terrestris*). The females' diet was complemented with standard reptile calcium supplements (Microcalcium TerraVit, JBL, Neuhofen, Germany) and vitamins (Nekton-rep, Nekton, Pfrozheim, Germany) to provide the nutrients required for egg development. Light and heat were provided by 40 W bulbs (photoperiod: 10 L : 14 D cycle) and ultraviolet (UV) lamps provided UVB and UVA for 1.5 h per day to facilitate calcium metabolism. Every morning and every night, terraria were sprayed with water to maintain high humidity conditions and were inspected for laid clutches. Clutches were placed into a small recipient filled with fine, water-saturated river soil and were incubated at 21°C during the day (09.00–21.00 h) and 19°C at night [29]. Immediately after hatching, we measured the body size (SVL, to the nearest 1 mm) of each hatchling and determined its sex using ventral scale counts [30]. Tissue samples (tail tip) from each hatchling and all unhatched eggs were conserved in 70% ethanol. All hatchlings were codified by toe clipping and released into the maternal population within 24 h after hatching. Released offspring were recaptured at the beginning of October 2009 to estimate survival selection during maturation.

(c) Paternity analyses

Paternity analyses based on four polymorphic microsatellite DNA loci (Lv-3–19, Lv-4–72, Lv-4-alpha, Lv-2–145; [31]) were used to measure morph siring success (i.e. the number of females with which a male fertilized at least one egg), the principal proxy on which sexual selection acts in *Z. vivipara* [32]. DNA from tissue samples and eggs was extracted using a BioSprint 96 DNA Blood Kit (Qiagen, Hilden, Germany). The methods used for polymerase chain reaction and determination of allelic size have been previously described [33]. The genetic profile of all mothers and of all potential fathers was known. Paternity assessment was performed manually and verified using CERVUS v. 3.0 [34]. In cases where two fathers matched the juvenile alleles, the paternity was confirmed by analysing two additional loci (Lv-4-X, Lv-4–115; [31]). All offspring were attributed without any mismatch to a single father except for six unhatched eggs in which the DNA was degraded.

(d) Statistics

Male morphs were modelled as described by Sinervo *et al.* [16] using two colour scores (o and y) that accounted for the number of putative colour alleles in males, as indicated in the Introduction. We used generalized linear mixed models (lme4 package, R 2.10.1) with a Poisson distribution to study RPS phase effects on siring success of male morphs and used a binomial distribution to study the effects of male siring success on offspring survival. All mixed models were fitted using maximum-likelihood (ML) estimation, and population was nested within RPS phase and modelled as a random factor. The full models used to analyse siring success included RPS phase, o and y scores and their interactions, and male body size as a covariate. Full models of offspring survival included RPS phase, morph siring success and offspring sex (and all their interactions) as fixed effects; offspring mother as random factors; and offspring body size and hatching date as covariates. Final models were obtained by backward elimination of non-significant ($p > 0.10$) interactions and verified by re-adding the excluded terms one at a time. To determine the robustness of the applied tests, all results were verified using bootstrap methods based on 1000 replicates ('pbkrtest' package, R 2.10.1). These analyses yielded qualitatively identical results in all cases and are not reported here.

The degree of mating discrimination (m) was calculated as the number of mate partners of the most successful morph
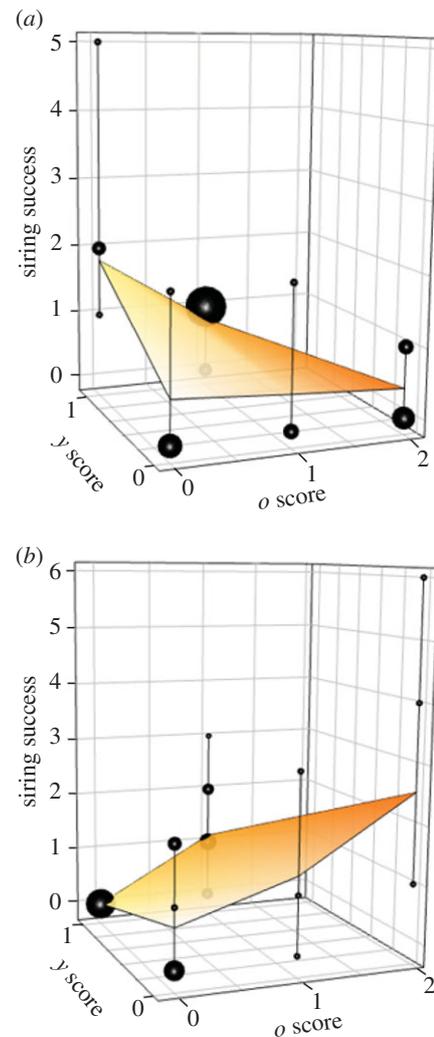
Figure 2. Siring success of male morphs with respect to RPS phase. Morph siring success (number of females with which a male morph sired offspring) as a function of male colour morph for (*a*) populations cycling towards high *o* frequency and (*b*) populations cycling towards high *w* frequency. *o* and *y* scores represent the number of putative colour alleles (note that in autumn 2008 no *yy* males were found in the field). Dots represent observed values and dot size is proportional to the number of observed values. Surfaces represent predictions generated using the statistical model.

relative to the number of mate partners of the other morphs and was determined for each putative colour allele (assuming additivity) and each population [16]. The observed survival advantage of the preferred morph's progeny was calculated for each population and mean ($\pm$ s.e.) values were compared with the survival advantage determined using eqns 2–5 of Sinervo *et al.* [16] and the reported values: c, 0.25; k, 0.5; d, 4.

3. Results

Male siring success significantly depended on male colour morph and the colour morph frequency of maturing and mature males (RPS phase $\times$ *o* score: $\chi_1^2 = 13.58$, $p < 0.001$; RPS phase $\times$ *y* score: $\chi_1^2 = 7.90$, $p = 0.005$; RPS phase $\times$ *o* score $\times$ *y* score: $\chi_1^2 = 5.09$, $p = 0.024$; figure 2). In populations cycling towards a high *o* frequency, siring success increased with increasing *y* score (figure 2a) and siring success was highest and lowest in *wy* (mean $\pm$ s.e.: 2.5 $\pm$ 0.89 females) and *oo* (0.42 $\pm$ 0.24 females) males, respectively. By contrast, in populations cycling towards high *w* frequency, siring success
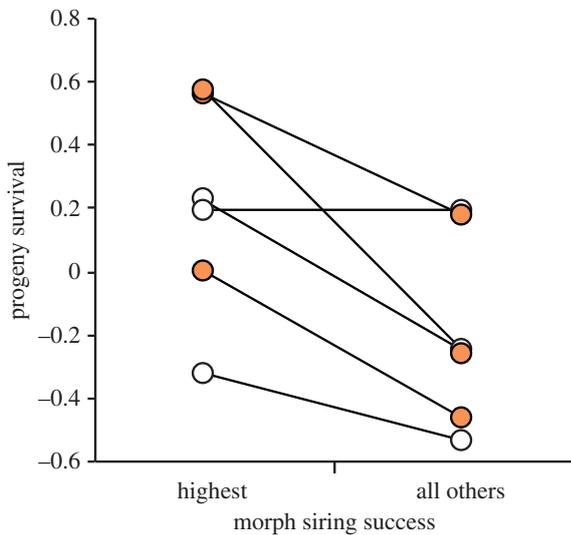
Figure 3. Progeny survival of colour morphs with respect to siring success. Survival of offspring sired by the male morph with the highest siring success (*wy* males in populations cycling towards *o* and *oo* males in populations cycling towards *w*; figure 2) and of offspring sired by the remaining morphs. Stars and associated error bars indicate mean ± s.e. per group. The survival probabilities for each of the two morph groups and for each of the six populations are shown. Lines connect survival probabilities measured in the same population. Populations cycling towards high *o* frequency are depicted with orange dots, and those cycling towards high *w* frequency with white dots.

increased with increasing *o* score and decreased with increasing *y* score (figure 2*b*). Siring success was lowest in *wy* males (no *wy* male sired eggs) and highest in *oo* males (3.67 ± 1.45 females). Body size positively affected the siring success ($\chi_1^2 = 16.62$, $p < 0.001$), in line with previous reports [35], and its effect was independent of RPS phase (body size × RPS phase: $\chi_1^2 = 0.05$, $p = 0.81$). The degree of sexual selection (i.e. the degree of mating discrimination, *m*; [16,36]) observed was 1.61 ± 0.15, which is above the minimum value of 1.3 predicted by a theoretical model and required to produce rapid RPS dynamics [16].

To test the prediction that survival probability is highest for the progeny of the most successful morph [16], we compared the survival of the offspring of the most successful morph (*wy* males in populations cycling towards *o*, and *oo* males in populations cycling towards *w*; figure 2) with that of the offspring of the remaining morphs. The survival of the offspring of the morph with the highest siring success was significantly higher than that of the offspring of the other morphs (estimate ± s.e.: 1.52 ± 0.62, $\chi_1^2 = 6.50$, $p = 0.011$; figure 3). The interaction between RPS phase and siring success was not significant ($\chi_1^2 = 0.36$, $p = 0.55$). Survival also depended on the offspring's sex ($\chi_1^2 = 4.31$, $p = 0.038$), hatching date ($\chi_1^2 = 24.76$, $p < 0.001$) and on body size at birth ($\chi_1^2 = 3.36$, $p = 0.067$). The observed survival advantage of the preferred morph's progeny was 1.14 ± 0.09, and thus within the range ($1.08–1.16$, mean ± s.e.: 1.10 ± 0.03) required to generate 3–4-year RPS cycles [16].

4. Discussion

Rapid morph frequency cycles resulting from RPS dynamics have been documented in short-lived species, where

frequency changes from one generation to the next require relatively low levels of negative FD selection [3]. RPS games may thus be restricted to a narrow parameter space and to specific evolutionary scenarios and ecological conditions [12,16]. By contrast, in the majority of species and especially in multi-annual iteroparous species with major generational overlap, only strong negative FD selection can produce substantial frequency bias that leads to rapid morph frequency cycles [16]. In the absence of strong FD selection morph frequency cycles may occur, but will at best have a very long periodicity (more than 16 generations) given that adult, maturing and juvenile cohorts continuously mix, which dilutes the frequency bias in the offspring's generation [4,17]. Here, we experimentally show that two cumulative FD and temporally distinct mechanisms (FD sexual selection and FD survival selection) acting at different stages of the life cycle, can cumulatively generate sufficient FD selection to produce rapid morph frequency cycles in species with major generational overlap. As predicted, FD sexual selection acted on male colour morph (prediction i), sexual selection depended on the morph frequency (prediction ii) and more specifically on the adult morph frequency encountered by progeny at birth and not on the adult frequency during sexual selection (prediction iii), sexual selection was reinforced by a second temporally distinct selective episode (prediction iv) and the degree of selection imposed was in the range of the degree of selection required to generate rapid morph frequency cycles in multi-annual iteroparous species with well-structured age classes (prediction v).

Here, we show that in populations cycling towards a high *o* frequency, siring success increased with increasing *y* score (figure 2*a*) and siring success was highest and lowest in *wy* and *oo* males, respectively. By contrast, in populations cycling towards high *w* frequency, siring success increased with increasing *o* score, decreased with increasing *y* score, and was highest and lowest in *oo* and *wy* males, respectively (figure 2*b*). These results are in line with prediction (i); that adult colour morph is sexually selected, and with prediction (ii); that sexual selection depends on male colour morph frequency. To test prediction (iii), that sexual selection depends on the adult morph frequency encountered by progeny at birth and not on the adult frequency during sexual selection, we manipulated the frequency of the maturing cohort and the colour morph frequency of adult males was held constant among RPS phases (see 08 frequency of *w* and *o* biased populations in figure 1*c*). During the breeding season in early spring, maturing males can be easily distinguished from adult and mature males owing to their smaller body size. In low altitude populations (as those simulated in our population experiment), maturing lizards reach adult body size and become mature between June and August, when juveniles hatch. If sexual selection depends on the frequency of the adult males present during sexual selection, then no differences between experimental RPS phases would be predicted, because the same adult colour morph frequency was established in both RPS phases, because no morph-specific survival differences in adult males existed between RPS phases before the breeding season (colour score × RPS phase: all $p > 0.11$), and because maturing lizards were not yet mature. In contrast to this prediction, there existed clear differences in sexual selection among RPS phases and sexual selection was with respect to the adult morph frequency encountered by progeny at birth in July/August, i.e. the

morph frequency of the released adult and maturing males. Thus, prediction (iii) was met.

Sexual selection may arise from inter- or intrasexual selection and in many species FD sexual selection is the consequence of intraspecific competition among males [4,8]. Likewise, in the classic RPS game played by *U. stansburiana* negative FD selection arises owing to intrasexual competition among males [4,8]. In our study, sexual selection was not the result of intrasexual selection among adult males, as there existed no colour morph frequency differences between RPS phases in adult males (see above). Moreover, it is as very unlikely that intrasexual selection among males, through inter-cohort competition among adult and maturing males, was the cause of the observed sexual selection, as adult males dominate smaller maturing males [37]. This indicates that differences in sexual selection among RPS phases cannot be explained by intrasexual selection among males of different cohorts, nor by intrasexual selection among males in general, which contrasts to findings in other species (e.g. in *U. stansburiana*; [4]). By contrast, the observed sexual selection is congruent with intersexual selection, and with the prediction that context-dependent female mate choice maximizes the progenies survival [16] (prediction iii), since in both RPS phases offspring produced by the most successful males (during sexual selection) survived best. In populations cycling towards *o*, the most successful morph during sexual selection was *wy*, and in populations cycling towards *w*, *oo* males were most successful. The survival probability of the progeny of the most successful morph (during sexual selection) was higher than that of the progeny of other morphs (figure 3) and this trend was observed in both RPS phases (note: there was no significant interaction between morph type (most successful morph versus all other morphs) and RPS phase). The observed morph-specific survival selection was thus consistent with prediction (iv), that sexual selection is reinforced by a second temporally distinct selective episode. This finding is, to our knowledge, the first robust evidence for the existence of two cumulative and temporally distinct episodes of FD selection. Moreover, both the degree of sexual selection (m) and the observed survival advantage of the preferred morph's progeny were above the minimum value required to generate rapid morph frequency cycles in RPS games driven by cumulative FD selective episodes [16]. The degree of selection imposed by the two cumulative selective episodes was thus in line with the magnitude of FD selection required to generate rapid 3–4 year cycles in multi-annual iteroparous species with well-structured age classes and thus with prediction (v).

In summary, our experiment provides, to our knowledge, the first evidence for the existence of multiple, complex and cumulative selective episodes, suggesting that the necessary conditions for the evolution of rapid RPS dynamics are much less restrictive than predicted by initial studies in short-lived species. This suggests that conditions suitable for the evolution of RPS dynamics may exist in almost all species, and thus that RPS dynamics may be more widespread than previously thought. The fact that two different, cumulative and temporally distinct episodes generated the FD selection required for the evolution of rapid RPS dynamics as well indicates that the evolution of rapid interspecific RPS dynamics is more likely, since the required FD selection might result from cumulative events that can be temporally distinct, e.g. FD selection produced by intra- and interspecific mechanisms. This indicates that beside their suggested importance for speciation [38], evolution of genetic architecture [39] and maintenance of genetic diversity [8], RPS dynamics may also affect ecological parameters ranging from dispersal [40,41], interspecific competition (e.g. host–parasite interactions; [42]), and density dependence to community structures and biodiversity [5,13,43].

References

1. Maynard Smith J. 1982 *Evolution and the theory of games*. Cambridge, UK: Cambridge University Press.

2. Nowak MA. 2006 *Evolutionary dynamics: exploring the equations of life*. Cambridge, MA: Harvard University Press.

3. Sinervo B, Calsbeek R. 2006 The developmental, physiological, neural, and genetical causes and consequences of frequency-dependent selection in the wild. *Annu. Rev. Ecol. Evol. Syst.* **37**, 581–610. (doi:10.1146/annurev.ecolsys.37. 091305.110128)

4. Sinervo B, Lively CM. 1996 The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **380**, 240–243. (doi:10.1038/ 380240a0)

5. Reichenbach T, Mobilia M, Frey E. 2007 Mobility promotes and jeopardizes biodiversity in rock-paper-scissors games. *Nature* **448**, 1046–1049. (doi:10.1038/nature06095)

6. Bleay C, Comendant T, Sinervo B. 2007 An experimental test of frequency-dependent selection on male mating strategy in the field. *Proc. R. Soc. B* **274**, 2019–2025. (doi:10.1098/rspb.2007.0361)

7. Kerr B, Riley MA, Feldman MW, Bohannan BJM. 2002 Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature* **418**, 171–174. (doi:10.1038/nature00823)

8. Zhang R, Clark AG, Fiumera AC. 2013 Natural genetic variation in male reproductive genes contributes to nontransitivity of sperm competitive ability in *Drosophila melanogaster*. *Mol. Ecol.* **22**, 1400–1415. (doi:10.1111/mec.12113)

9. Svensson E, Abbott J, Härdling R. 2005 Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. *Am. Nat.* **165**, 567–576. (doi:10.1086/429278)

10. Shuster S, Wade M. 1991 Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350**, 608–610.

11. Agren J, Ericson L. 1996 Population structure and morph-specific fitness differences in tristylous *Lythrum salicaria*. *Evolution* **50**, 126–139. (doi:10. 2307/2410787)

12. Zamudio KR, Sinervo B. 2003 Ecological and social contexts for the evolution of alternative mating strategies. In *Lizard social behavior* (eds SF Fox, TA Baird, JK McCoy), pp. 83–106. Baltimore, MD: Johns Hopkins University Press.

13. Buss LW, Jackson JBC. 1979 Nontransitive competitive relationships in cryptic coral reef environments. *Am. Nat.* **113**, 223–234.

14. Mills SC, Hazard L, Lancaster L, Mappes T, Miles D, Oksanen TA, Sinervo B. 2008 Gonadotropin hormone modulation of testosterone, immune function, performance, and behavioral trade-offs among male morphs of the lizard *Uta stansburiana*. *Am. Nat.* **171**, 339–357. (doi:10.1086/527520)

15. Sinervo B, Miles DB, Frankino WA, Klukowski M, DeNardo DF. 2000 Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Horm. Behav.* **38**, 222–233. (doi:10.1006/hbeh.2000.1622)

16. Sinervo B, Heulin B, Surget-Groba Y, Clobert J, Miles DB, Corl A, Chaine A, Davis A. 2007 Models of density-dependent genic selection and a new rock-paper-scissors social system. *Am. Nat.* **170**, 663–680. (doi:10.1086/522092)

17. Sinervo B, Zamudio KR. 2001 The evolution of alternative reproductive strategies: fitness differential, heritability, and genetic correlation between the sexes. *J. Hered.* **92**, 198–205. (doi:10.1093/jhered/92.2.198)

18. Strijbosch H, Creemers RCM. 1988 Comparative demography of sympatric populations of *Lacerta vivipara* and *Lacerta agilis*. *Oecologia* **76**, 20–26.

19. Shine R, Charnov EL. 1992 Patterns of survival, growth, and maturation in snakes and lizards. *Am. Nat.* **139**, 1257–1269.

20. Massot M, Clobert J, Montes-Poloni L, Haussy C, Cubo J, Meylan S. 2011 An integrative study of ageing in a wild population of common lizards. *Funct. Ecol.* **25**, 848–858. (doi:10.1111/j.1365-2435.2011.01837.x)

21. San-Jose LM, Granado-Lorencio F, Sinervo B, Fitze PS. 2013 Iridophores and not carotenoids account for chromatic variation of carotenoid-based coloration in common lizards (*Lacerta vivipara*). *Am. Nat.* **181**, 396–409. (doi:10.1086/669159)

22. Sinervo B *et al.* 2006 Self-recognition, color signals, and cycles of greenbeard mutualism and altruism. *Proc. Natl Acad. Sci. USA* **103**, 7372–7377. (doi:10.1073/pnas.0510260103)

23. Arribas O. 2009 Morphological variability of the Cantabro-Pyrenean populations of *Zootoca vivipara* (Jacquin, 1787) with description of a new subspecies. *Herpetozoa* **21**, 123–146.

24. Fitze PS, Le Galliard J-F. 2008 Operational sex ratio, sexual conflict and the intensity of sexual selection. *Ecol. Lett.* **11**, 432–439. (doi:10.1111/j.1461-0248.2008.01158.x)

25. Heulin B, Osenegg-Leconte K, Michel D. 1997 Demography of a bimodal reproductive species of lizard (*Lacerta vivipara*): survival and density characteristics of oviparous populations. *Herpetologica* **53**, 432–444.

26. Massot M, Clobert J, Pilorge T, Lecomte J, Barbault R. 1992 Density dependence in the common lizard: demographic consequences of a density manipulation. *Ecology* **73**, 1742–1756.

27. Mugabo M, Perret S, Legendre S, Le Galliard J-F. 2013 Density-dependent life history and the dynamics of small populations. *J. Anim. Ecol.* **86**, 1227–1239. (doi:10.1111/1365-2656.12109)

28. Fitze PS, Cote J, Clobert J. 2010 Mating order-dependent female mate choice in the polygynandrous common lizard *Lacerta vivipara*. *Oecologia* **162**, 331–341. (doi:10.1007/s00442-009-1463-1)

29. Heulin B, Arrayago MJ, Bea A, Braña F. 1992 Caractéristiques de la coquille des oeufs chez la souche hybride (ovipare × vivipare) du lézard *Lacerta vivipara*. *Can. J. Zool.* **70**, 2242–2246.

30. Lecomte J, Clobert J, Massot M. 1992 Sex identification in juveniles of *Lacerta vivipara*. *Amphibia-Reptilia* **13**, 21–25.

31. Boudjemadi K, Martin O, Simon J-C, Estoup A. 1999 Development and cross-species comparison of microsatellite markers in two lizard species, *Lacerta vivipara* and *Podarcis muralis*. *Mol. Ecol.* **8**, 518–520.

32. Fitze PS, Le Galliard J-F. 2011 Inconsistency between different measures of sexual selection. *Am. Nat.* **178**, 256–268. (doi:10.1086/660826)

33. Laloi D, Richard M, Lecomte J, Massot M, Clobert J. 2004 Multiple paternity in clutches of common lizard *Lacerta vivipara*: data from microsatellite markers. *Mol. Ecol.* **13**, 719–723. (doi:10.1046/j.1365-294X.2004.02102.x)

34. Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**, 639–655. (doi:10.1046/j.1365-294x.1998.00374.x)

35. Fitze PS, Cote J, Martínez-Rica JP, Clobert J. 2008 Determinants of male fitness: disentangling intra- and inter-sexual selection. *J. Evol. Biol.* **21**, 246–255. (doi:10.1111/j.1420-9101.2007.01447.x)

36. Alonzo SH, Sinervo B. 2001 Mate choice games, context-dependent good genes, and genetic cycles in the side-blotched lizard, *Uta stansburiana*. *Behav. Ecol. Sociobiol.* **49**, 176–186. (doi:10.1007/s002650000265)

37. Richard M, Lecomte J, De Fraipont M, Clobert J. 2005 Age-specific mating strategies and reproductive senescence. *Mol. Ecol.* **14**, 3147–3155. (doi:10.1111/j.1365-294X.2005.02662.x)

38. Corl A, Davis AR, Kuchta SR, Sinervo B. 2010 Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proc. Natl Acad. Sci. USA* **107**, 4254–4259. (doi:10.1073/pnas.0909480107)

39. Sinervo B, Svensson E. 2002 Correlational selection and the evolution of genomic architecture. *Heredity* **89**, 329–338. (doi:10.1038/sj.hdy.6800148)

40. Sinervo B, Clobert J. 2003 Morphs, dispersal behavior, genetic similarity, and the evolution of cooperation. *Science* **300**, 1949–1951. (doi:10.1126/science.1083109)

41. Sinervo B, Calsbeek R, Comendant T, Both C, Adamopoulou C, Clobert J. 2006 Genetic and maternal determinants of effective dispersal: the effect of sire genotype and size at birth in side-blotched lizards. *Am. Nat.* **168**, 88–99. (doi:10.1086/505765)

42. Cameron DD, White A, Antonovics J. 2009 Parasite-grass-forb interactions and rock-paper-scissor dynamics: predicting the effects of the parasitic plant *Rhinanthus minor* on host plant communities. *J. Ecol.* **97**, 1311–1319. (doi:10.1111/j.1365-2745.2009.01568.x)

43. Frean M, Abraham ER. 2001 Rock-scissors-paper and the survival of the weakest. *Proc. R. Soc. B* **268**, 1323–1327. (doi:10.1098/rspb.2001.1670)

7

rspb.royalsocietypublishing.org *Proc. R. Soc. B* **281**: 20140976