

Experimental design

Aluminium chamber bases were pressed 10 cm into the sediment to isolate square patches of sea floor 50 × 50 cm. Sixteen chamber bases were established in close proximity at a depth of 6 m. *Echinocardium* were either added or removed from the bases to create experimental treatments with 0, 4, 8 or 16 urchins per chamber. The following morning, chamber lids (8 clear and 8 opaque) with non-directional water stirrers were fitted, enclosing about 25 l of bottom water. The light–dark treatment was interspersed equally across the urchin density treatment in a randomized block design. Chamber water was sampled near midday at 1.5–2 h intervals (exact times noted in each instance), providing the raw data for flux calculations 24 h after *Echinocardium* density manipulations. Bottom water external to the chambers was also sampled at each interval, and light and dark bottles were established just above the seabed at time = 0 on the day of sampling. Dissolved oxygen was measured within minutes of collection (YSI model 5730 BOD bottle probe) and water samples were filtered immediately thereafter (1.1 µm pore size Whatman GF/C glass fibre filter). All samples were kept in darkness, and stored frozen until analysis.

Water chemistry

Analysis for ammoniacal nitrogen (NH₄-N), nitrate-plus-nitrite nitrogen (NO_x-N) and dissolved reactive phosphorus (DRP) used standard methods for sea water²⁹ on an AlphKem series 500 air-segmented continuous flow auto-analyser; detection limits <0.1 µmol l⁻¹ for N and P.

Sediments and macrofauna

To characterize features of the sediment column that could potentially affect pore water chemistry and flux chamber measurements, several types of sediment samples were collected from each chamber at the end of each experiment. Two surface sediment samples (~30 g scrapes to 2 cm depth) were collected, one for chlorophyll *a* analysis and one for organic matter content and sediment texture analysis. Chlorophyll *a* was extracted from sediment by boiling in 95% ethanol and analysed spectrophotometrically³⁰. Organic matter content was assessed by % sediment mass lost following combustion (% loss on ignition, LOI). Sediment texture was assessed by standard sieve and pipette techniques after removal of organic matter (digestion in 9% hydrogen peroxide). Water content in the upper 5 cm of sediment was determined from one 2.4 cm diameter core per chamber from the difference in sample wet weight and dry weight. Macrofauna were collected with one 10 cm diameter, 13 cm deep core taken from the centre of each chamber base. Macrofauna were sieved on a 500 µm mesh sieve and preserved in 70% isopropyl alcohol + rose bengal for later sorting and identification. When all sediment samples had been collected, the entire area enclosed by each chamber base was excavated to a depth of approximately 5 cm in order to quantify all *Echinocardium* present at time = end.

Data analysis

Each experiment was analysed separately. Sediment and faunal variables were used as predictors of dissolved chemical fluxes in multiple regression models. Variables were eliminated by backward selection unless significant at α = 0.15. Collinearity among predictor variables was avoided by examining variance inflation factors and condition indices. Homogeneity of variance was evaluated by plotting residuals versus predicted values, and normality was assessed via normal probability plots and Shapiro-Wilk tests on residuals, though no data transformations were required.

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Effect of extrinsic mortality on the evolution of senescence in guppies

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Classical theories^{1,2} for the evolution of senescence predict that organisms that experience low mortality rates attributable to external factors, such as disease or predation, will evolve a later onset of senescence. Here we use patterns of senescence in guppies derived from natural populations that differ in mortality risk to evaluate the generality of these predictions. We have previously found that populations experiencing higher mortality rates evolve earlier maturity and invest more in reproduction, as predicted by evolutionary theory³. We report here that these same populations do not have an earlier onset of senescence with respect to either mortality or reproduction but do with respect to swimming performance, which assesses neuromuscular function. This mosaic pattern of senescence challenges the generality of the association between decreased extrinsic mortality and delayed senescence and invites consideration of more derived theories for the evolution of senescence.

Medawar's¹ 'mutation accumulation' theory predicts that populations with high mortality rates should accumulate deleterious mutations that reduce fitness late in life. This age specificity occurs

because few individuals survive long enough to experience the purifying effects of natural selection that would otherwise remove late-acting mutations from the gene pool. Williams² 'antagonistic pleiotropy' theory predicts that high mortality rates will select for earlier maturity and a higher rate of investment in reproduction early in life, which incurs a cost in the form of reduced investment in maintenance and reproduction late in life. More derived theories for the evolution of senescence generate variations on these predictions. For example, age classes may vary in their susceptibility to extrinsic mortality⁴ or there may be an interaction between senescence and vulnerability to mortality factors, such that senescence selectively increases the probability of death in one subset of the population more than others^{5,6}. Increases in extrinsic mortality rate may be accompanied by decreases in population density and increases in resource availability to survivors⁵. When such complexities are included, increased extrinsic mortality may cause the evolution of earlier senescence, later senescence, or no change in patterns of senescence, depending on these additional factors.

There is a striking discrepancy between the diversity of theory on the evolution of senescence and its treatment in the literature. Empirical evaluations of the evolution of senescence focus almost exclusively on the classical theory⁷⁻¹⁶, as do recent reviews^{17,18}.

Here we report a comparative study of senescence based on the quantification of complete life histories of 240 individually reared guppies (*Poecilia reticulata*) derived from high and low mortality rate environments in Trinidad. High mortality rate sites are streams where predators co-occur with guppies. These predators are often excluded from the upper reaches of streams by waterfalls, giving rise to low mortality rate sites. Predators increase the mortality rates of all size/age classes of guppies, and guppies cannot outgrow susceptibility to predation. The probability of surviving for six months in the low predation sites is 20–30 times greater than in high predation sites^{19,20}.

We compared senescence among paired high and low predation

localities from two drainages, the Oropuche and Yarra. Previous research suggests that guppies have adapted to high and low predation environments independently in each drainage²¹. Our comparisons were made between second-generation, laboratory reared offspring derived from wild-caught females. Two generations of lab rearing removes confounding environmental effects. The offspring are the product of a breeding design that equally represents 25 wild-caught adult females, so they are free of adaptation to the laboratory or inbreeding²² (see Methods). Fish were reared individually, beginning at an age of 25–30 days and ending at death. Data collected included the age at first reproduction, age and size at all subsequent reproductive events, number of offspring and age at death. In order to compare populations in an environment as free as possible of extrinsic mortality that comes as close as possible to replicating nature, we matched water quality, temperature and photoperiod to prevailing conditions in nature, following the methods of our earlier work²².

We reared the fish at two levels of food availability to match average differences among high and low predation localities in nature²². Low predation localities tend to have lower levels of food availability²³, reflected in the lower growth rates and smaller asymptotic body sizes of guppies from those sites. Our high and low food treatments yielded asymptotic body sizes that approximated what we observed in high and low predation environments (data not shown). Thus, the complete factorial experiment was a comparison of guppies from high and low mortality rate environments in two drainages, for a total of four localities, crossed with two levels of food availability.

Whereas theory is clear in making predictions about how senescence should evolve, it is less clear about how one should quantify senescence. We have taken literally the definition of senescence as any age-specific decline in variables associated with individual fitness, specifically mortality, reproduction and physiological performance.

High predation guppies matured at a significantly earlier age, reproduced more frequently and produced more offspring in each litter²⁰, which is consistent with our earlier studies^{3,22}. Guppies provide no postnatal care for their young, so the age at last reproduction is equivalent to dying with respect to the determination of an individual's fitness. Because analyses of age at last reproduction and age at death yield qualitatively similar results, we

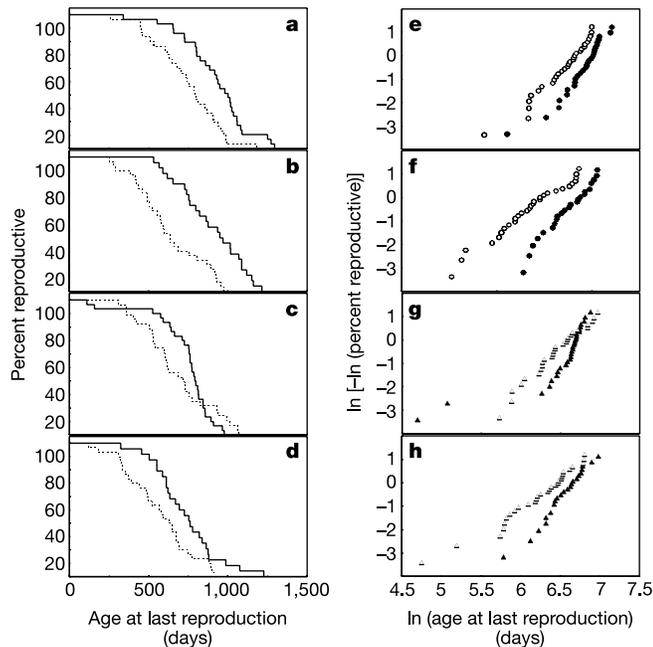


Figure 1 Age at last reproduction. **a–d**, The proportion of the population that is still reproducing at a given age (y -axis) plotted against the age at last reproduction (x -axis). High predation (solid line), low predation (stippled line). **e–h**, The natural log of negative natural log of the percent of fish that are still reproducing (y -axis) plotted against the age at last reproduction (natural log transformed, x -axis). High predation (filled symbols), low predation (open symbols). Oropuche, high food (**a, e**). Oropuche, low food (**b, f**). Yarra, high food (**c, g**). Yarra, low food (**d, h**).

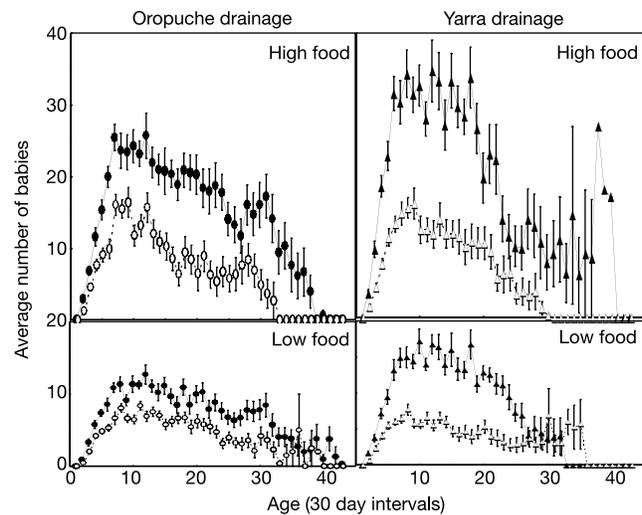


Figure 2 Fecundity. Number of offspring (y -axis) produced per 30 days (age, x -axis). We report the average number (± 1 s.e.m.) of babies produced by each treatment group per 30 days. The magnitude of the standard errors increases and fecundity becomes more erratic with increasing age because the sample sizes decline. Oropuche (circles), Yarra (triangles), high predation (filled), low predation (open).

summarize here results for only age at last reproduction (Fig. 1; for age at death see Supplementary Information).

First, we compared the fit of a Gompertz versus an exponential function to the data (S-Plus, *CensorReg* routine)²⁴. The Gompertz function provided a significantly better fit to the data in all comparisons (likelihood ratio test, $P < 10^{-4}$; Supplementary Information) indicating that mortality rate increases with age in all treatment groups.

Initial analyses of the age at last reproduction, assuming a Gompertz distribution, showed a significant interaction between drainage and predation, so we analysed drainages separately. High and low predation sites differ significantly in both drainages (Oropuche: z -value = -2.76 , $P = 0.0057$; Yarra: z -value = -3.23 , $P = 0.0012$). There was also a significant effect of food in the Oropuche drainage ($z = 2.00$, $P = 0.0459$) and a significant food by predation interaction in the Yarra drainage ($z = 2.61$, $P = 0.0091$). Food effects reflect the longer reproductive life spans of low food fish in the high and low predation sites from the Oropuche drainage and the low predation site from the Yarra drainage (Table 1).

The rate of ageing¹³ is lower in the high predation localities in all four paired comparisons (drainage by food) between guppies from high and low predation localities (Table 1). This index is a function of the α and γ parameters of the Gompertz equation [$m_x = \alpha e^{\gamma x}$] and hence of what is interpreted as the baseline mortality rate and the rate of increase in mortality with age²⁵. This result reflects the lower rates of cessation of reproduction throughout the lives of the high predation fish (Fig. 1, details and alternative indices of senescence in Supplementary Information).

Guppies from high predation localities begin reproduction at an earlier age²⁰, cease reproduction at a later age (Fig. 1) and hence have a longer reproductive lifespan. They also have longer total life spans (Table 1) but there are no differences among treatment groups in post-reproductive lifespan (data not shown). The differences in lifespan are thus concentrated in the duration of reproduction. These results do not conform with the conventional prediction that the populations from low mortality rate environments will also evolve delayed senescence if senescence is assayed as reproductive lifespan or rate of ageing¹³. We obtained similar results for guppies from a third pair of populations in a small pilot study that preceded this experiment (Supplementary Information).

A second way of evaluating senescence is through changes in the rate of production of offspring with age. Guppies from high predation localities in both drainages sustained a higher rate of

production of offspring throughout their lives (Fig. 2). Litter size is positively correlated with female size, so offspring production per unit time increased for seven to eight months after maturity in association with continued growth of the mother, then levelled off as females attained asymptotic body size. All treatment groups then experienced a progressive decline in offspring production with age, which is consistent with reproductive senescence.

The distribution of age-specific fecundity, which is the number of offspring produced per unit time, is triangular in shape and can be described with a modification of the fecundity function suggested by McMillan *et al.*²⁶. The key parameters are the age at which fecundity is greatest (D_{max}) then the rate of decline in fecundity (b). The age at maximum fecundity was significantly later in low food treatments and in fish from the Oropuche drainage (see Methods). There was a non-significant trend ($P = 0.107$) for guppies from high predation communities to attain peak fecundity at a later age than those from low predation communities. The rate of decline in fecundity with age is only affected significantly by drainage ($P < 10^{-4}$); it is steeper in the Yarra drainage (Table 1). We obtained similar results with an alternative analysis in which we fit a linear regression to fecundity on age for ages beyond D_{max} (b_{lin} , Table 1) (Supplementary Information). Although not significant, the rate of decline in fecundity estimated as b_{lin} is always lower in guppies from high predation localities. Whereas these analyses do not provide statistical support for differences among predator communities in reproductive senescence, they also do not comply with the prediction for delayed senescence in guppies from low predation communities. If anything, the trends are in the opposite direction. A similar analysis of reproductive value, which is a composite measure of age-specific mortality and reproduction, yields qualitatively similar results (Supplementary Information).

Finally, we used the fast start escape response as an assay of neuromuscular performance. This response is a stereotyped burst of movement used to evade a striking predator. Peak accelerations during fast starts can be up to 40 body lengths s^{-2} (ref. 27). Age-related declines in the neuromuscular system have been documented as a cause of reduced physical performance²⁸. Because the fast start reflects a fish's neuromuscular performance, it serves as an index of physiological senescence. We estimated the maximum acceleration for a subset of our fish at an age of approximately 12 months then again at an age of approximately 26 months. There was a significant deceleration in all fish as they aged ($F_{1,35} = 36.85$, $P < 0.0001$), and a significant interaction between age and predation ($F_{1,35} = 5.6$, $P = 0.0237$) because guppies from high predation

Table 1 Life history statistics by treatment group and parameter estimates for the triangular fecundity function

Drainage	Food	Predation	Total life span (days)*	Age at last reproduction (days)*	Rate of ageing ¹³ (ω)	Number of litters*	Number of offspring*	Estimated parameters of the triangular fecundity function and b_{lin} .					
								D_{max}	M_{∞}	k	t_0	b	b_{lin}
Oropuche	High	High	1006.6 (636–1,264)	883.2 (533–1,216)	0.0047	27.7 (14–43)	545.4 (204–1,114)	10.42	3.61	0.47	1.07	0.010	–0.026
		Low	746.4 (338–1,136)	615.3 (251–976)	0.0117	16.8 (5–32)	217.6 (18–568)	9.57	4.17	0.46	0.99	0.015	–0.045
	Low	High	1030.9 (434–1,464)	914.9 (340–1,294)	0.0048	27.6 (7–38)	258.8 (51–444)	10.52	2.69	0.50	1.42	0.010	–0.025
		Low	844 (461–1,230)	730.4 (255–1,181)	0.0083	20.5 (5–29)	138.3 (17–212)	10.78	3.31	0.39	0.99	0.015	–0.037
Yarra	High	High	850.8 (448–1,228)	724.9 (324–1,228)	0.0100	25.6 (11–43)	607.7 (169–1,114)	9.12	2.12	0.30	0.79	0.039	–0.039
		Low	699.4 (164–1,291)	553.3 (115–908)	0.0131	15.7 (2–32)	204.6 (6–476)	8.59	3.73	0.41	1.07	0.035	–0.075
	Low	High	803 (151–1,075)	728.2 (109–976)	0.0035	25.1 (2–35)	283.5 (5–467)	10.78	2.49	0.27	0.67	0.027	–0.030
		Low	788.2 (396–1,253)	667.6 (306–1,066)	0.0110	18.5 (8–31)	115.0 (9–278)	9.09	1.29	0.33	0.79	0.029	–0.044

D_{max} equals the time interval in which fecundity was greatest. M_{∞} = the potential maximum daily fecundity (log transformed). t_0 = the first day of offspring production. k characterizes the rate of increase in fecundity. b and b_{lin} characterize the rate of decrease in fecundity with age. *mean (range).

environments experience a more rapid deterioration in physiological performance with age than do their counterparts from low predation environments (Fig. 3). In separate analyses of each age, high predation guppies were significantly faster than low predation guppies when they were young ($F_{1,35} = 5.64, P = 0.0231$) but the two groups did not differ significantly when they were old ($F_{1,35} = 0.66, P = 0.42$).

Our results do not comply with the classical Medawar–Williams theory when senescence is evaluated in terms of survival, fecundity or reproductive value. Guppies from high predation localities have lower rates of ageing and do not differ in reproductive senescence relative to those from low predation localities. The only aspect of our results that complies with predictions is our assay of performance. Why did the unexpected happen? First, prior empirical support for these predictions is limited. In support of the classical theory, research on *Daphnia*¹⁴, opossums⁹ and grasshoppers¹² all found that populations with lower mortality rates had delayed senescence. In addition, Stearns *et al.*¹⁵ successfully selected for lower intrinsic mortality rates in *Drosophila melanogaster* that experienced lower extrinsic adult mortality rates.

There are three exceptions to the classic predictions based on extrinsic mortality that can contribute to our unexpected results. Williams proposed that “senescence should be more rapid in those organisms that do not increase markedly in fecundity after maturity than those that do show such an increase”²². If fecundity increases with age, then this increase can offset the age-specific decline in fitness caused by mortality. In nature, guppies from high predation localities grow faster and attain larger asymptotic body sizes than those from low predation localities²³. Because litter size is directly proportional to body size, their faster growth translates into a higher rate of increase in fecundity with age (Supplementary Information). A consequence of this increase in fecundity is that it should offset some of the differences in mortality rate between high and low predation localities and hence result in smaller differences in senescence than expected from mortality rate alone²⁹.

Charlesworth⁴ and Abrams⁵ show that how organisms evolve in response to extrinsic mortality depends upon whether or not populations are subject to density regulation. To paraphrase one prediction, predators increase mortality rate by eating prey, but may decrease mortality rates indirectly by reducing density and increas-

ing per capita resource availability. If older age classes benefit more than younger age classes from higher resource availability, then higher mortality can cause the evolution of delayed senescence, even though increased mortality without an indirect effect of density predicts the evolution of earlier senescence. A diversity of other responses is possible, depending on the effects of density on the survival of different age classes and fecundity. Such indirect effects of predation occur in natural populations of guppies²³. Furthermore, the significant interaction between food availability and predation in the Yarra replicate argues that resource availability can affect the evolution of senescence; however, we do not yet have sufficient knowledge of the nature of density regulation in guppies to formally test these theories. There has already been a definitive demonstration in *Drosophila melanogaster* that ecology can alter the evolution of senescence. Luckinbill and Clare³⁰ showed that selection on late-life reproductive success causes the evolution of later senescence if larval density is high, but has no effect on the evolution of senescence if larval density is low. Stearns *et al.*¹⁵ successfully selected for later senescence and the evolution of other life history traits by decreasing adult mortality rates, but only after increasing larval density and decreasing food supply.

Abrams⁵, and Williams and Day⁶ present a third alternative by considering that the risk of mortality due to factors like disease or predation is often dependent on the condition of the individual. For example, if an age-related decline in escape response (physiological senescence) increases the risk of predation, then predation will select for improved ability to evade predators. A consequence of selection for improved escape performance will be deferred senescence in the age classes that have high reproductive potential. The strength of such selection should decline as an individual’s reproductive potential declines with age. At the same time, the costs of deferred senescence will accumulate. Predation can thus select for a lower rate of senescence early in life but accelerated senescence late in life⁶. Our finding that high predation guppies have faster rapid-start responses early in life suggests that predators select for improved escape performance. The predicted lower rate of senescence early in life but more rapid senescence later in life is consistent with the trend in both drainages for high predation guppies to have a delayed, but then more rapid, acceleration in the cessation of reproduction and mortality (Fig. 1, Supplementary Information) relative to low predation guppies.

All three alternatives have properties that are applicable to guppies and all may contribute to our unexpected results. More generally, the classical prediction that high extrinsic mortality will cause the evolution of earlier senescence has dominated our thinking because it makes intuitive sense and has not been challenged by discordant empirical data. Our results provide new incentive to consider the importance of the derived models for the evolution of senescence. □

Methods

Localities and lab rearing: adult females were collected from the Yarra and Oropuche drainages in April, 1998. The approximate grid references for each collection, read from 1:25,000 topographic maps, are: Yarra River, PS940(N) PS804(W); Yarra Tributary(Limon River), PS876(N) PS834(W); Oropuche River, PS788(N) QS043 (W); Oropuche Tributary (Campo River), PS813 (N) PS971(W). The first generation of laboratory-born offspring were born between May and July, 1998, then were reared to adulthood and mated to produce the second generation of laboratory born offspring in November, 1998. Ten young produced by these crosses were reared in two groups of five in 8 l aquaria to an age of 25–30 days, then were sexed and two females were selected from each litter for inclusion in the senescence assay (initiated in January and February 1999). Females were reared by themselves in 8 l aquaria and were mated beginning one week after entering the assay—five weeks before the minimum age at first parturition. All other aspects of rearing were as in our previous studies. Survival at all stages was generally >95%, which is part of the basis for arguing that there is little or no inadvertent selection associated with this breeding design.

Statistics

To calculate survival we used the Censor Reg routine in S-Plus with the extreme value distribution²⁴. The evaluation of the Gompertz function and the associated slopes and

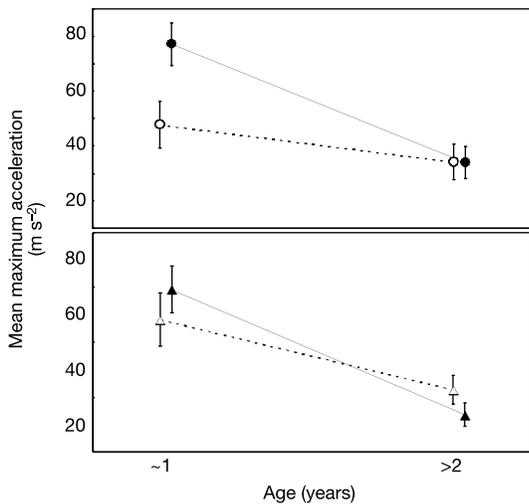


Figure 3 Maximum acceleration. The average maximum acceleration (± 1 s.e.m.) during the induced alarm response (y-axis) is plotted against age (x-axis) with the results for each drainage presented separately. Oropuche high predation (filled circles), Oropuche low predation (open circles), Yarra high predation (filled triangles), Yarra low predation (open circles).

intercepts of the fitted curves can be obtained as functions of the extreme value distribution as described in the S-Plus Guide to Statistics²⁴.

Fecundity was calculated using our modification of the triangular fecundity function²⁶, takes the form:

$$\ln(M_x) = M_\infty (1 - e^{-(kx+t_0)})e^{-bx} \quad (1)$$

where M_x is the fecundity at age x , and the parameters M_∞ , k , t_0 and b were fitted by minimizing the sums of squares. M_∞ is the potential maximum daily fecundity (log transformed). t_0 is the first time period of offspring production, where each unit of time equals 30 days. k characterizes the rate of increase in fecundity at age x whereas b characterizes the rate of decrease in fecundity at age x . At early ages the term $(1 - e^{-(kx+t_0)})$ dominates and accounts for the rise in fecundity, whereas at later ages the term e^{-bx} dominates and largely determines the rate of decline in fecundity. The age at which fecundity is greatest, D_{\max} , is given by

$$D_{\max} = \frac{1}{k} \left(\ln \left(\frac{b+k}{b} \right) + t_0 \right) \quad (2)$$

Although b is a measure of the decline in fecundity, its value is also influenced by the increasing phase of the fecundity curve. A second measure of the rate of decline that is independent of the increasing phase is a linear regression of fecundity on age for ages beyond D_{\max} ; we refer to the slope of this regression as b_{lin} (Supplementary Information). We used D_{\max} for statistical comparisons among treatment groups of the age at maximum fecundity and b and b_{lin} for the rate of decline in fecundity with age. Both b and b_{lin} differed significantly among drainages; b_{lin} also differed among food levels. Both categories of variables can serve as indices of senescence. Standard errors for the estimates were obtained by the delete-one jackknife³¹ (Supplementary Information). To remove heteroscedasticity we used the rank-transformed values of b . The pseudovalues from the jackknife procedure were used to test for variation due to drainage, food ration, predation type and all interactions.

Performance was evaluated for 9–13 fish from each population at both ages. Food effects were not significant after performance was size corrected and was not included in the analysis. Fish were placed in a glass tank with a 1 cm² reference grid on the bottom. This focal tank was in turn placed within a larger glass tank that helped regulate water temperature. Fast-starts were elicited then filmed at 500 frames per sec (Redlake Motionscope camera) and recorded to VHS videocassette tape. Recorded sequences were converted from analogue (VHS) to digital format and saved as AVI files. The dorsal midlines of the first three tail beat cycles were digitized using a modification of the public domain NIH Image program (available at <http://rsb.info.nih.gov/nih-image/>) for the Apple Macintosh. We fit a cubic spline function to each digitized midline within a sequence and used this function to find the coordinates of an unmeasured landmark at 0.35 total length from the tip of the head, which we used as a proxy for the centre of mass. We used a MSE quintic spline algorithm to smooth the displacement versus time data and to estimate instantaneous velocities and accelerations throughout a digitized sequence. From the fitted functions, we computed maximum accelerations occurring within the first 22 ms. The analysis was a repeated measures Anova with predators, drainage and time as main effects. Drainage and interactions between drainage and other variables were not significant. Maximum acceleration was the dependent variable. See ref. 32 for additional details.

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Population density drives the local evolution of a threshold dimorphism

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Evolution can favour more than one reproductive tactic among conspecifics of the same sex^{1,2}. Under the conditional evolutionarily stable strategy, individuals adopt the tactic that generates the highest fitness return for their status: large males guard females, whereas small males sneak copulations^{3,4}. Tactics change at the status at which fitness benefits switch from favouring one tactic to favouring the alternative^{1,5}. This ‘switch-point’ is expressed in many species as a threshold between divergent morphologies³. Environmental and demographic parameters that influence the relative fitness of male tactics are predicted to determine a population’s switchpoint^{1,5} and consequently whether the population is monomorphic or dimorphic. Here we show threshold evolution in the forceps dimorphism of the European earwig *Forficula auricularia* and document the transition from completely monomorphic to classical male-dimorphic populations over a distance of only 40 km. Because the superior fighting ability of the dominant morph⁶ will be more frequently rewarded at high encounter rates, population density is likely to be a key determinant of the relative fitness of the