

An integrative study of ageing in a wild population of common lizards

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Summary

1. Integrative studies on ageing patterns in multiple traits of organisms are challenging and rare in free-living populations. However, developing integrative approaches could prove useful to understanding ageing patterns as causes of age variations are diverse, with conflicting or related actions. Accordingly, we investigated age variation of multiple aspects in the common lizard *Lacerta (Zootoca) vivipara*.

2. In a wild population of common lizards, we studied five fitness components, three physiological traits (cell-mediated immunity, corticosterone level, resting metabolic rate), and controlled for individual and environmental heterogeneities. To quantify ageing patterns in fitness, we used individual-based data collected over 14 years (18 684 captures, 892 reproductive events).

3. Ageing patterns were found in multiple aspects. They provided evidence for female maturation early in adulthood (access to reproduction, litter size), followed by senescence in female reproduction (litter success) and survival. In parallel to senescence, a pattern of terminal investment enhanced offspring quality (offspring body size, offspring corpulence, litter success). Ageing patterns involved physiology with higher metabolic rate and T cell-mediated immune response in old females.

4. Several ageing patterns were dependent on environmental and individual characteristics (habitat, year, sex, body size). Interestingly, senescence occurred only in females with a high reproductive effort early in life. Rarely showed, this trade-off between early and late-life performances is expected under the antagonistic pleiotropy and disposable soma theories of senescence.

5. Overall, this study emphasizes the interest of integrative studies to investigate the multifaceted process of ageing.

Key-words: corticosterone, fitness, immunity, maturation, metabolic rate, reptile, senescence, terminal investment

Introduction

Many organisms show age variation in life-history traits and individual performances. Age variations may be because of a wide range of processes that are often thought to interact (e.g. negative relationships between early and late performances in life, higher reproductive performances in old individuals with a low life expectancy) (Stearns 1992). It is therefore essential that ageing processes are investi-

gated simultaneously. Such studies are challenging in free-living populations as they require the study of multiple traits on a large number of individuals of known age.

Ageing processes may be individual or populational. There are three true individual ageing processes not related to the selective appearance or disappearance of individuals in populations (Forslund & Part 1995): maturation, terminal investment, and senescence. First, performance often increases with age in early adulthood (Forslund & Part 1995). This maturation process may be because of improvements in physiology (developmental maturation), morphology (larger body size), or behaviour (learning from experience, change in social

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status) (Forslund & Part 1995; Bowen *et al.* 2006; Low, Part & Forslund 2007; Sparkman, Arnold & Bronikowski 2007).

Secondly, individual performance may increase with age later in adulthood. It is classically defined as the hypothesis of the 'residual reproductive value' or 'terminal investment': the reproductive effort should increase when the reproductive value of individuals declines with age so as to maximize lifetime reproduction (Stearns 1992; Descamps *et al.* 2007). This duality between the reproductive effort and reproductive value complicates studies (Catry *et al.* 2006; Descamps *et al.* 2007) and could explain why terminal investment is so rarely documented.

Thirdly, senescence corresponds to irreversible deteriorations in physiological condition in old individuals which leads to lower longevity or/and reproduction. Three non-mutually exclusive theories have been proposed as evolutionary causes of senescence. The mutation accumulation theory holds that deleterious mutations amass with age because of a decline in the force of selection in older ages (Medawar 1952; Hamilton 1966). Based on the same idea of a decline of selection with age, the antagonistic pleiotropy theory proposes that senescence results from pleiotropic genes with both positive effects early in life and detrimental effects later on (Williams 1957). The disposable soma theory, a specific type of pleiotropy, emphasizes the importance of the trade-off between reproduction and somatic maintenance (Kirkwood 1977). The disposable soma and antagonistic pleiotropy theories expect negative relationships between early and late fitness components in adulthood based on the cost of reproduction early in life, but very few studies have tested for this delayed cost of reproduction (Nussey *et al.* 2006). The evolutionary theories of senescence also expect the fitness decline to start after reproductive maturity. However, senescence may be delayed in species where adults continue to grow and increase their fecundity (Vaupel *et al.* 2004).

Integrative studies testing for multiple factors could greatly improve our knowledge of ageing in wild populations (Bronikowski & Promislow 2005; Lecomte *et al.* 2010). First, the study of multiple factors increases the chance to detect non-exclusive processes contributing to the same ageing variation (Forslund & Part 1995; Low, Part & Forslund 2007; McCleery *et al.* 2008) or with conflicting actions (Catry *et al.* 2006). Secondly, it is interesting to investigate multiple traits because their age variation may differ (Bronikowski & Promislow 2005; Nussey *et al.* 2008; Lecomte *et al.* 2010). Thirdly, the small number of old individuals in natural populations makes it difficult to have a high statistical power (McCleery *et al.* 2008; Nussey *et al.* 2008), and a low statistical evidence may be reinforced by several variables showing the same trend. In particular, investigating multiple variables could provide greater information on the still debated issue of the form of senescence in wild animals (Nussey *et al.* 2008).

Here we performed an integrative study of age-specific variations by collecting physiological and fitness data in a wild population of the common lizard *Lacerta (Zootoca) vivipara* (Fig. 1). We also tested for interactions with environmental and individual heterogeneities, notably the



Fig. 1. Common lizard adult female. How its survival, reproductive performances and physiology vary with age is investigated in this study. Photograph M. Massot.

reproductive effort early in life. Furthermore, the variability that can be associated with different environmental conditions and taxonomic groups makes it a priority to perform field studies on diverse organisms (Monaghan *et al.* 2008; Robert & Bronikowski 2010). Ageing patterns in the wild are predominantly documented in birds and mammals (Jones *et al.* 2008), and very little is known in other taxa (Bonduriansky & Brassil 2005). Reptiles have been the subject of few studies (Patnaik 1994; Congdon *et al.* 2003; Robert & Bronikowski 2010), and these do not provide enough evidence for generalization.

Materials and methods

SPECIES

Lacerta vivipara is a lacertid lizard widely distributed across Europe and Asia, and is usually found in peat bogs and heath lands. It is a live-bearing species, except in the extreme south of Europe where oviparous populations have been recorded (Surget-Groba *et al.* 2001). In our study region (southern France, 44°30'N 3°45'E, 1420 m a.s.l.), hibernation lasts from October to April. Males emerge from hibernation in April, and mating occurs soon after female emergence in early May. Parturition occurs usually between mid-July and mid-August. Average litter size is of five shell-less eggs, and females give birth to fully formed juveniles that emerge from the foetal membrane within 1 or 2 h after laying. There is no parental care, and juveniles are independent from birth. The activity season ends in late September.

POPULATION MONITORING

The study population consists of two contiguous zones that differ for structural diversity of habitats with regard to relative abundances in heath and grass patches, rocks and trees (Clobert *et al.* 1994): F+ area (4300 m²) has a higher structural diversity than F- area (4700 m²). Lizard life-history traits also differ between habitats: F+ has higher lizard densities (700 vs. 430 adults ha⁻¹ in F-), F+ hatchlings have smaller body sizes, and F+ females lay earlier (Clobert *et al.* 1994). Dispersal between the two zones is *c.* 10% of juveniles, and less than 5% of adults. We collected reproductive and capture-recapture data from 1989 to 2002. Many individuals were of known age because they were identified either as juveniles (born in the current year) or yearlings (born the year before). At the first capture, individuals

Table 1. Numbers of adult males and females with a known age collected in the two zones of the study site (F+, F-) from 1989 to 2002 at the Mont Lozère (France)

Age (years)	2	3	4	5	6	7	8	9	10	Total
F+ males	226	51	29	20	13	1	0	0	0	340
F- males	78	32	7	3	1	0	0	0	0	121
F+ females	262	167	92	63	26	7	1	0	1	619
F- females	91	72	34	24	13	4	1	0	1	240
Total	657	322	162	110	53	12	2	0	2	1320

The table reports a single occurrence of each individual with the oldest age recorded.

were toe-clipped, with no effect on subsequent recapture and survival probabilities (Massot *et al.* 1992). We identified 8758 individuals from 18 684 captures during the 14 study years, and age-specific sample sizes per study zone in adult males and females are given in Table 1. Data collection each year was structured as follows: a capture session of yearlings and adults in June, a temporary transfer of pregnant females to the laboratory in July to survey reproductive outputs and mark juveniles from birth, and a capture session of juveniles in September. During each capture session, we measured, weighed and identified individuals directly in the field, and immediately released them at their point of capture. Except in years 1998 and 2002, we also recorded the reproductive status of adult females in June (mated females exhibiting mating scars). Pregnant females captured in July were transferred to a field laboratory until parturition (usually between mid-July and mid-August). The females were housed in plastic terraria (18 × 12 × 12 cm) under standardized conditions (Massot & Clobert 2000). After giving birth, each female was weighed and juveniles were measured, weighed and sexed. We then released females and juveniles at the female's capture point.

PHYSIOLOGICAL MEASUREMENTS

We investigated the age variation of T lymphocyte cell-mediated immunity which is one of the main components of immunity in vertebrates. This is the most commonly immune variable tested in wild animals, and it may have fitness consequences because it constitutes a generalized response to allergens and wounds (Norris & Evans 2000). We evaluated T cell-mediated immunity, 1 day after parturition, of 51 females [15: 2 year old (y/o), 16: 3 y/o, 9: 4 y/o, 6: 5 y/o, 5: older than 5 years] in 2005 and 2006. The T cell proliferation was assessed by a subcutaneous injection of a mitogen (phytohaemagglutinin PHA) and a measurement of the swelling that occurred 12 h later. The right leg was injected with 0.04 mL of a solution containing 2.5 mg of phytohaemagglutinin in 1 mL phosphate buffered saline (PBS). The left leg was injected with 0.04 mL of PBS. The reaction to the PHA injection, in terms of leg thickening, was assumed to be proportional to the intensity of the T lymphocyte cell-mediated immunity (Martin, Scheuerlein & Wikelski 2003). Larger localized swelling indicates a more robust immune response. To assess the T-cell response, we measured the thickness of the left and right legs using a spessimeter (Mitutoyo ID-C112, Kawasaki, Japan) with an accuracy of 0.01 mm. After 12 h later the thickness of both legs was measured again at the inoculation point. Reaction to PHA was expressed as the difference between the changes in thickness of both legs (Meylan, Haussy & Voituron 2010).

We measured the corticosterone level of 50 females in 2004 (22: 2 y/o, 14: 3 y/o, 9: 4 y/o, 2: 5 y/o, 3: older than 5 years). Blood samples (40–80 µL whole blood) were collected from the post-orbital sinus using 2–3 50 µL microhematocrit tubes. Blood sampling was

conducted in females 2 days after parturition. All samples were collected between 16:00 and 17:30 h, and within 2 min of removal of an animal from its home cage to avoid a handling effect (Dauphin-Villemant & Xavier 1987). The initial rise of the corticosterone level due to captivity disappears after a few days (Dauphin-Villemant & Xavier 1987; Meylan, Dufty & Clobert 2003). Blood samples were centrifuged and the plasma harvested and stored at –20 °C until transported on dry ice to our laboratory in Paris, where they were maintained at –80 °C. Corticosterone levels were assessed using a competitive enzyme immunoassay with Octeia corticosterone kit (IDS, Fountain Hills, AZ, USA) (Fitze *et al.* 2009).

In 2006 and 2007, we measured the resting metabolic rate defined as the minimum rate of energy expenditure during the activity period of the daily cycle (Andrews & Pough 1985). We measured 39 females (16: 2 y/o, 12: 3 y/o, 4: 4 y/o, 4: 5 y/o, 3: older than 5 years). We measured the oxygen consumption using an open air flow respirometer from Sable Systems (Las Vegas, NV, USA) comprising a two-channel pump PP-2, two mass flow controller electronics units MFC-2, an eight-channel multiplexer TR-RM8, an FC-10a Oxygen analyzer, and a subsampler/pump/mass flow meter unit TR-SS3. We placed individuals in a 200 mL dark chamber, with a 20 mL min⁻¹ airflow. The consumptions was recorded every 1 min for 30 min, with the temperature maintained at 25.0 ± 0.5 °C. The volume of oxygen consumed was calculated from the difference between the oxygen level in the ambient air and that at the exit of the chamber, and we used the minimal value. Lizards were deprived of food for 3 days before measurements, and placed inside the chamber at least 1 h before the beginning of the observations. Our measurement was the volume of dioxygen consumed per mass and time units ($r = 0.78$, $n = 39$, $P < 0.0001$ for the relationship between the metabolic rate and body mass).

STATISTICAL ANALYSES

Survival estimates were obtained independently of capture probabilities using capture–mark–recapture methods based on the open population model of Cormack–Jolly–Seber. This model produces 'apparent survival estimates' that result from mortality and emigration. Because we showed that emigration is rare in our study population, our estimates may be assimilated to true survival rates (Massot *et al.* 1992). We performed survival analyses on individuals of known age, and with capture–recapture histories starting at a given age to model a true age effect (Clobert, Lebreton & Allaine 1987). We used the program MARK (White & Burnham 1999) to fit models, and models were compared with Akaike Information Criterion (AIC). It is usually considered that two models differ when the difference in AIC between the two models is higher than 2 (White & Burnham 1999). The best model is the most consistent with the data while using the fewest number of parameters, i.e. giving the lowest AIC. The good-

ness-of-fit of the time-dependent Cormack–Jolly–Seber model was tested with the bootstrap procedure (1000 simulations) provided by the program MARK (White & Burnham 1999), and we did not find significant over-dispersion of the data ($P = 0.4556$).

We performed longitudinal analyses (analyses on repeated individual measurements) to test age dependence in reproductive performances. We used Generalized Linear Mixed Models (MIXED procedure of SAS, version 9.1; SAS Institute Inc., Cary, NC, USA) that are of great interest in the study of ageing in the wild because they provide a statistical method of disentangling true ageing effects from individual heterogeneity between ages (Nussey *et al.* 2006, 2008). Models included age, study zone, and body size (snout-vent length: svl) as fixed factors, first-order interactions with age (age \times svl, age \times zone), and individual as a random effect. Because age effects are often curvilinear, squared term of age was also included in models. As we did not have repeated measures for the physiological variables, they were analysed using ANCOVAs. In all these analyses, we selected final models after backward elimination of independent factors with $P > 0.10$, and we checked residuals of the initial models for normality and homoscedasticity.

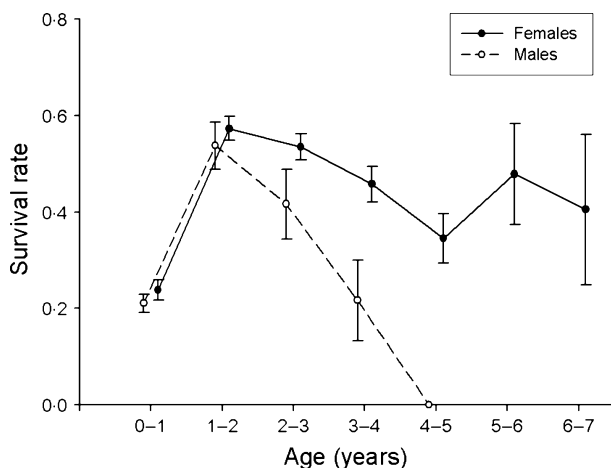


Fig. 2. Age-specific survival rates of males and females. Estimates and standard errors come from 2146 juvenile males and 1927 juvenile females for survival rates between birth and 1 year (label 0–1), from 879 yearling males and 841 yearling females for survival rates between 1 and 2 years (label 1–2), and from 366 two-year-old males and 610 two-year-old females for adult survival rates.

Results

SURVIVAL RATES

Age- and sex-specific survival rates from birth to adult ages are given in Fig. 2. We found that after the classical improvement in survival until maturity (usually at 2 years in our population), the survival probabilities decreased with age. This ageing pattern was faster in males than in females ($\chi^2_1 = 7.18$ $P = 0.0074$). Thereafter, we focused analyses on adult females because we did not have data on the reproductive performances of males. In order to better characterize the decrease in adult female survival rates, we performed detailed analyses on 610 adult females of known age by comparing models in which survival rates and recapture probabilities were dependent on year, age (class effect, linear trend, quadratic relationship, two categories of age) or were constant. The best models were dependent on year for recapture probabilities (Table 2) as previously found (Massot *et al.* 1992). For survival rates, we found four equivalent models, all corresponding to a decrease in survival with age (Table 2, Fig. 2). Selecting one of the four different models of survival decrease (linear, quadratic or threshold effect after age of 3 or 4 years) would require a large amount of supplementary capture–recapture data. However, another possibility is to investigate other variables as reported below.

REPRODUCTIVE PERFORMANCES OF FEMALES

Female age at maturity of *L. vivipara* is known to depend on body size, year, density, and population (Massot *et al.* 1992; Richard *et al.* 2005). Thus, we tested age variation in reproductive status (pregnant vs. non-pregnant females) with logistic regression controlling for body size, year, and the two study zones. Reproduction of females was function of age ($\chi^2_9 = 77.23$ $P < 0.0001$), and we found a clear cut-off between 2 and 3 years of age. All females of 3 years of age and older were observed as pregnant ($n = 378$), while 18.7% of 2-year-old (y/o) females were non-pregnant ($n = 310$). We found a yearly variation in the reproductive status of 2 y/o females ($\chi^2_{11} = 90.89$ $P < 0.0001$) that was greatly, but not only, explained by body size variation in these young

Table 2. Survival analysis in adult females

Survival									
Recapture	t	a	A	A^2	2 years vs. ≥ 3 years	2–3 years vs. ≥ 4 years	2–4 years vs. ≥ 5 years	2–5 years vs. ≥ 6 years	Constant
t	1501.12	1500.17	1491.32	1492.61	1491.70	1492.22	1499.37	1499.24	1500.72
a	1520.08	1515.09	1510.60	1512.53	1513.52	1511.09	1514.49	1512.63	1514.66
Constant	1517.39	1518.52	1506.13	1507.33	1506.36	1507.72	1514.99	1514.97	1514.64

Table reports AICc (Akaike Information Criterion corrected for small sample size, White & Burnham 1999) for different year- (time effect t) and age-dependent models of survival and recapture probabilities. Age dependence was modelled as different age classes (a), linear trend (A) or quadratic variation (A^2). The other models compared two age classes at different thresholds (e.g. 2–3 years vs. ≥ 4 years contrasted yearly survival rates of 2- and 3-year-old females with survival rates of older females). The lower the AICc value is the more appropriate the model to fit the data. The lowest values of AICc, comparable with values which do not differ more than 2, are reported in bold.

females ($\chi^2_{11} = 21.41$ $P = 0.0294$ for year effect in the model including body size; $\chi^2_1 = 38.02$ $P < 0.0001$ for body size effect, $\chi^2_1 = 0.60$ $P = 0.4399$ for study zone effect). Non-reproducing 2 y/o females were smaller females with a snout-vent length threshold of 56 mm in June (95.6% of non-pregnant females were smaller than 56 mm).

We investigated other age-specific reproductive performances (see Table 3) with Generalized Linear Mixed Models including individual as a random effect, age (linear and quadratic relationships), body size (svl), and study zone as fixed factors. Because we searched for variability of age-specific responses, we also tested age \times svl and age \times zone interactions. Litter size significantly varied with age in a quadratic function ($P = 0.0016$), with increases throughout young ages (Fig. 3a). In addition, the slope of the positive relationship between female body size and litter size was significantly weaker in younger females (Fig. 3a) as indicated by the interaction between age and body size ($P = 0.0486$). The litter success was linearly dependent on age ($P = 0.0211$), the proportion of live neonates decreasing with female age (Fig. 3b). We performed analyses on neonatal characteristics of offspring through their body size and corpulence (residuals from body mass–svl relationship). Overall, we observed that neonatal characteristics were largely dependent on maternal age (Fig. 4). Corpulence increased with mother's age both in offspring males and females (Fig. 4c,d; $P = 0.0034$ in males, $P = 0.0010$ in females). Neonatal body size also varied with environmental conditions or mother's body size. Older mothers produced larger offspring males, but particularly in the study zone F– (Fig. 4a; $P_{\text{age}\times\text{zone}} = 0.0121$). Body size of offspring females was positively dependent on maternal age, but only in smaller mothers (Fig. 4b; $P_{\text{age}\times\text{svl}} = 0.0153$).

AGE-SPECIFIC PHYSIOLOGY

We investigated age variations in adult females for the circulating corticosterone level, immune cellular response and resting metabolic rate (see Table 4). Due to the lack of repeated measurements for the same females from different years, we only performed cross-sectional analyses that might reflect individual and/or population patterns. In addition, we completed our main analyses with specific tests on age thresholds as performed in the female survival analysis to increase our statistical power on relatively small sample sizes. Circulating corticosterone level did not differ between adult females of different ages ($P = 0.9621$, $n = 50$; see Table S1, Supporting information). Comparisons between age classes were also non-significant ($P > 0.0870$). In contrast, the immune response seemed dependent on age ($P = 0.0400$ for the comparison 2–4 y/o females vs. older females), although this relationship was not significant in the overall analysis ($P = 0.2297$, $n = 51$). The inflammatory response to the PHA injection tended to be higher in the oldest females (Fig. 5a). Finally, the metabolic rate of pregnant females was affected by their age differently depending on years ($P_{\text{age}\times\text{year}} = 0.0217$). The metabolic rate significantly

Table 3. Analyses on ageing variation of female reproductive performances

	Litter size	Litter success	Svl _m	Svl _f	Corp _m	Corp _f
Age	$F_{1,216} = 0.65$ $P = 0.4214$	$F_{1,364} = 5.37$ $P = 0.0211$	$F_{1,115} = 12.95$ $P = 0.0005$	$F_{1,104} = 6.90$ $P = 0.0099$	$F_{1,116} = 8.94$ $P = 0.0034$	$F_{1,106} = 11.53$ $P = 0.0010$
Age ²	$F_{1,216} = 10.22$ $P = 0.0016$	$F_{1,361} = 0.05$ $P = 0.8235$	$F_{1,112} = 2.03$ $P = 0.1566$	$F_{1,103} = 2.16$ $P = 0.1447$	$F_{1,112} = 0.07$ $P = 0.7961$	$F_{1,103} = 0.73$ $P = 0.3945$
Svl	$F_{1,216} = 0.35$ $P = 0.5527$	$F_{1,364} = 9.37$ $P = 0.0024$	$F_{1,114} = 0.01$ $P = 0.9062$	$F_{1,104} = 2.44$ $P = 0.1212$	$F_{1,115} = 0.54$ $P = 0.4642$	$F_{1,105} = 1.86$ $P = 0.1752$
Zone	$F_{1,155} = 0.23$ $P = 0.6310$	$F_{1,363} = 1.26$ $P = 0.2630$	$F_{1,93} = 3.71$ $P = 0.0573$	$F_{1,85} = 2.54$ $P = 0.1145$	$F_{1,93} = 1.92$ $P = 0.1690$	$F_{1,85} = 1.21$ $P = 0.2738$
Age \times svl	$F_{1,216} = 3.93$ $P = 0.0486$	$F_{1,360} = 0.02$ $P = 0.8859$	$F_{1,113} = 2.50$ $P = 0.1163$	$F_{1,104} = 6.09$ $P = 0.0153$	$F_{1,114} = 0.86$ $P = 0.3563$	$F_{1,104} = 1.58$ $P = 0.2122$
Age \times zone	$F_{1,212} = 0.15$ $P = 0.7017$	$F_{1,362} = 0.73$ $P = 0.3942$	$F_{1,115} = 6.50$ $P = 0.0121$	$F_{1,102} = 0.30$ $P = 0.5878$	$F_{1,113} = 0.22$ $P = 0.6389$	$F_{1,102} = 0.06$ $P = 0.8056$
Individual identity	$P = 0.0118$	$P = 0.7877$	$P = 0.2467$	$P = 0.1035$	$P = 0.4454$	$P = 0.0455$
S ² _{Ind}	41.8%	< 1.0%	39.0%	43.8%	2.4%	44.8%
N _{Females}	157	160	95	87	95	87
N _{Litters}	377	380	212	194	212	194

Analyses were mixed linear models (MIXED procedure of SAS, SAS Institute Inc.) on females with at least two reproductive events in the laboratory. Litter size, proportion of alive neonates (litter success), offspring male body size (svl_m), offspring female body size (svl_f), offspring male corpulence (corp_m), offspring female corpulence (corp_f) were standardized per year and modelled with the fixed factors age (linear effect), age² (quadratic age effect), svl (body size), and zone (study zone). We also included the first-order interactions with age and the individual identity random effect. Are reported the percentage of variance explained by the individual identity effect (S²_{Ind}), the number of females used in the analyses (N_{Females}), and the number of litters (N_{Litters}). Significant effects are in bold.

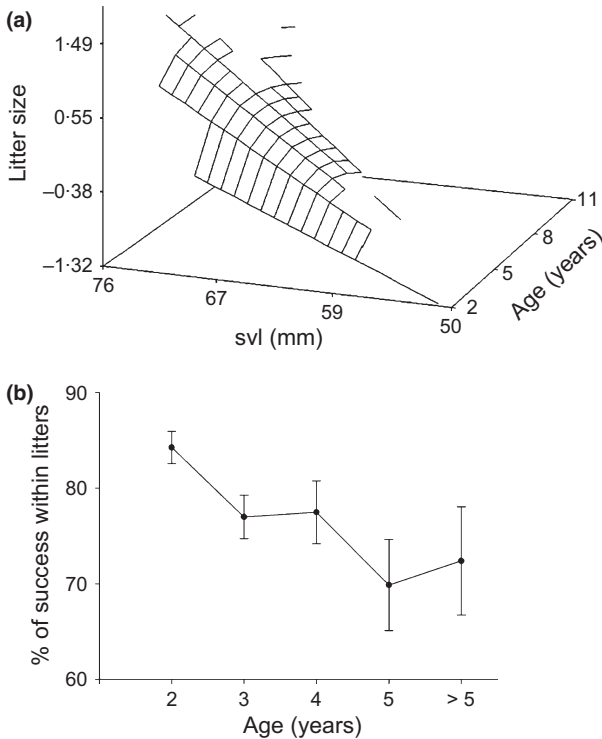


Fig. 3. Age-specific litter size and litter success. (a) Litter size in relation to age and body size (svl). The figure reports standardized predicted values from the final model reported in Table 3. Predicted values of litter size are only reported for non-missing combinations of age and svl. (b) Relationship between the percentage of alive neonates and female age.

increased with female age in 2006 ($P = 0.0322$, $n = 22$), but did not significantly vary with age in 2007 ($P = 0.1719$, $n = 17$) (Fig. 5b). The difference in metabolic rate between 2 and 4 y/o females and older females was marginally significant in 2006 ($P = 0.0549$), and not significant in 2007 ($P = 0.9669$).

REPRODUCTIVE EFFORT

Senescence may be because of the cost of reproduction early in life. Thus, we investigated the effect of the first reproductive effort when females were 2 years old. We defined the reproductive effort from residuals of the relationship between litter size and female body size ($r = 0.53$, $P < 0.0001$), categories of small and high reproductive efforts corresponding respectively to negative and positive residuals. The reproductive effort did not influence the ageing variation of litter size ($P = 0.7663$) and offspring body size ($P = 0.3352$ in females; $P = 0.4259$ in males). However, we found significant interactions between the reproductive effort and female age on the litter success (Fig. 6b; $P = 0.0287$), corpulence in offspring males (Fig. 6c; $P = 0.0006$) and females (Fig. 6d; $P = 0.0280$). While females that engaged in a small reproductive effort did not show an age variation on litter success ($P = 0.3227$), females that engaged in a large reproductive effort showed a significant increase of litter success with age ($P = 0.0331$). Offspring corpulence decreased in old females that engaged in a small reproductive effort, and increased with age in females that engaged in a large reproductive effort. These variations of corpulence were

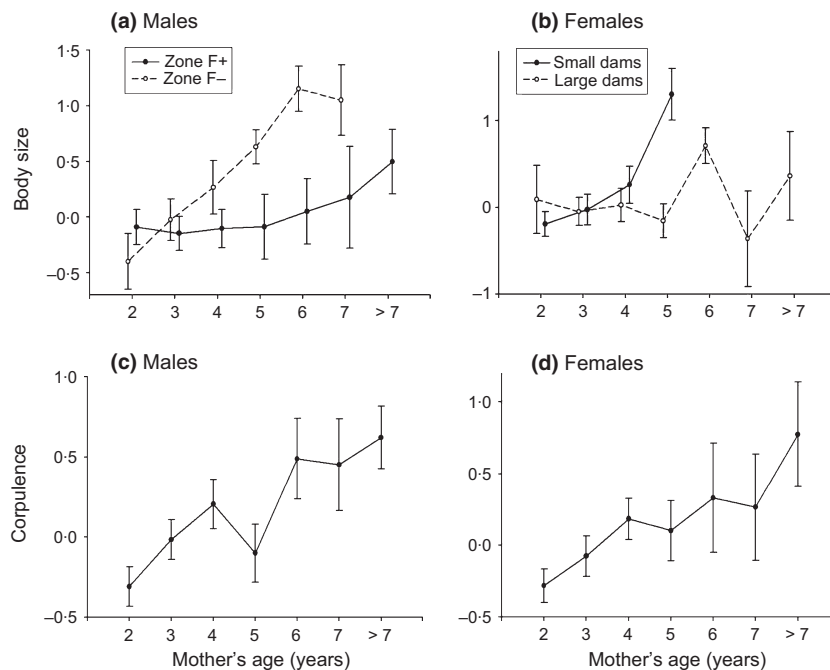


Fig. 4. Age-specific offspring characteristics at birth. Body size in offspring males (a) and females (b) are svl standardized per year. Their age variation respectively relates to study zone and mother's body size. Corpulence in males (c) and females (d) are residuals from body mass–svl relationship. Vertical lines are standard errors.

Table 4. Tests on ageing variation of adult female physiology

	PHA	Metabolic rate	Corticosterone
Age	$F_{1,49} = 1.48 P = 0.2297$	$F_{1,35} < 0.01 P = 0.9820$	$F_{1,47} = 0.44 P = 0.9621$
Age ²	$F_{1,43} = 0.46 P = 0.5019$	$F_{1,30} = 0.47 P = 0.4966$	$F_{1,46} = 0.57 P = 0.4542$
Svl	$F_{1,48} = 2.06 P = 0.1579$	$F_{1,32} = 0.07 P = 0.8005$	$F_{1,48} = 3.90 P = 0.0539$
Year	$F_{1,46} = 0.61 P = 0.4405$	$F_{1,35} = 0.87 P = 0.3579$	–
Zone	$F_{1,47} = 1.15 P = 0.2893$	$F_{1,34} = 0.09 P = 0.7605$	$F_{1,44} = 0.03 P = 0.8716$
Age × svl	$F_{1,44} = 0.08 P = 0.7734$	$F_{1,31} = 0.46 P = 0.5043$	$F_{1,45} = 0.23 P = 0.6366$
Age × year	$F_{1,45} = 0.82 P = 0.3697$	$F_{1,35} = 5.77 P = 0.0217$	–
Age × zone	$F_{1,42} = 0.12 P = 0.7324$	$F_{1,33} = 0.59 P = 0.4469$	$F_{1,43} = 0.25 P = 0.6170$

Effect of age (linear and quadratic effects), body size (svl), year and zone on the inflammatory response to PHA ($N = 34$ in 2005, $N = 17$ in 2006) and the resting metabolic rate ($N = 22$ in 2006, $N = 17$ in 2007). The similar analysis was performed on the corticosterone level ($N = 50$), but only on a single year (2004). Significant effect is in bold.

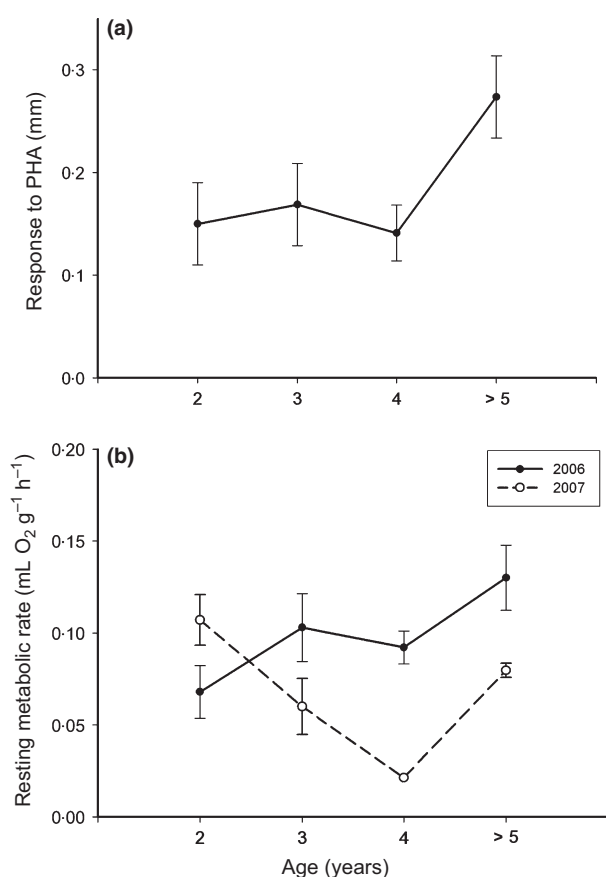


Fig. 5. Age-specific physiological characteristics in adult females. Figure reports means and standard errors. (a) Inflammatory response measured by the reaction to phytohaemagglutinin (PHA) of 51 females expressed as the difference between the change in thickness of the right PHA-inoculated leg and the change in thickness of the left leg. (b) Resting metabolic rate of adult females in 2006 ($n = 22$) and 2007 ($n = 17$). The similarity in age variations between the metabolic rate in 2006 and PHA response was not induced by a relationship between them ($r = 0.11$, $n = 16$, $P = 0.68$).

very similar in offspring males and females (Fig. 6c,d). We also tested the effect of the first reproductive effort on female survival by using capture–recapture data from our best models on cohorts of 2 y/o females (age-dependent survival rates

and year-dependent recapture probabilities). The ageing decrease of survival rates we previously found only characterized the females that engaged in a large reproductive effort in their first reproduction (Fig. 6a; $\chi^2_1 = 8.18 P = 0.0042$). Unlike the global survival analysis, it was possible to show here that the form of senescence was nonlinear. The two best models selected (with the smallest AIC that did not differ of more than 2) were the quadratic (AIC = 394.1) and the threshold effect after the age of 4 years (AIC = 393.5), compared to other shapes of age dependence (AIC = 397.6 for linear effect; AIC = 405.1 for the threshold effect after age of 3 years).

Discussion

Our long-term and integrative study on the common lizard revealed age variation in multiple aspects. These age variations involved physiology, reproductive performances, and survival. We found contrasting age variations with some fitness components that increase with age early in adulthood, some that increase later in life, and some others that decrease with age. Age-specific physiological traits observed (PHA, metabolic rate) might be the source of some of these age-specific fitness variations.

MULTIPLE AGEING PROCESSES

The causes of age variation are basically investigated from age-specific fitness components. Conclusions of studies could depend on the choice of fitness measurements. First, ageing variation may preferentially affect survival or reproduction as they are not under the same physiological and behavioural constraints. Ageing responses might also differ between reproductive traits related either to parental fitness or to offspring fitness. In addition, physiological determinants may vary between reproductive traits determined early (access to reproduction, litter size) and late (litter success, offspring quality) during the reproductive process. Using an overall estimate of fitness, combining its different components, could be a possibility to integrate this heterogeneity, and this is particularly adapted in comparative analyses from studies using slightly different fitness estimates (Jones *et al.* 2008). How-

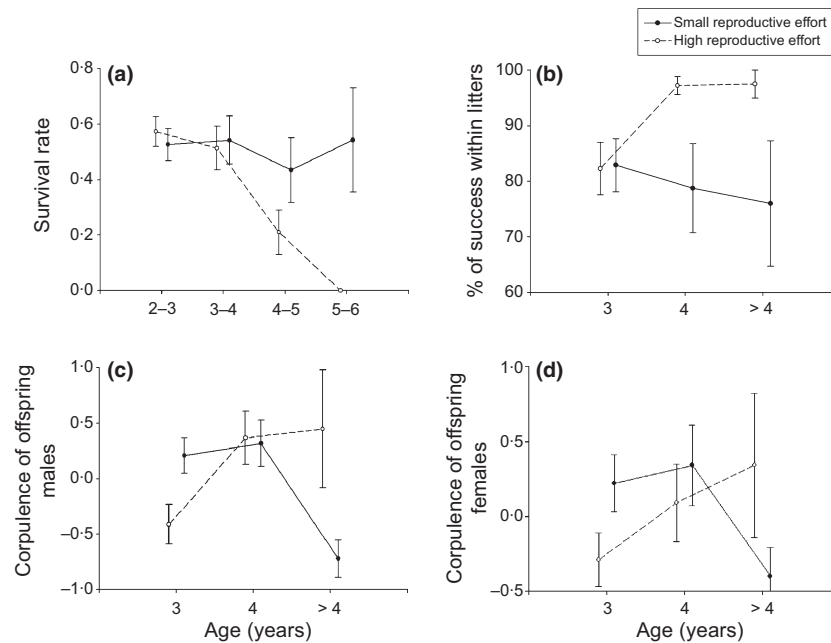


Fig. 6. Relationships between fitness components and the first reproductive effort. The reproductive effort was defined as small for negative residuals of the relationship between litter size and female body size, and as high reproductive effort for positive residuals. Vertical lines are standard errors. (a) Age-specific survival rates were obtained from cohorts of 128 and 162 two-year-old females, respectively for small and high reproductive efforts. (b) Average age-specific litter success corresponds to the percentage of alive neonates within litters in females with a small first reproductive effort ($n = 67$), and in females with a high first reproductive effort ($n = 69$). (c) Corpulence in offspring males are the residuals from body mass–svl relationship ($n = 45$ for small effort, $n = 49$ for high effort). (d) Corpulence in offspring females are the residuals from body mass–svl relationship ($n = 43$ for small effort, $n = 48$ for high effort).

ever, this approach masks the detection of multiple ageing processes acting simultaneously on different fitness components, and also ignores trade-offs that mediate interdependent responses between different fitness components. The studies on age variation are most often performed either on survival or on some reproductive performances. We chose to tackle age variation of fitness in a comprehensive approach based on multiple fitness components: survival, age at maturity, litter size, litter success, offspring quality (offspring body size, and corpulence).

We found that access to reproduction as well as litter size increase with age early in adulthood, and this corresponds to a reproductive maturation (Forslund & Part 1995). Female age at maturity is either at 2 or 3 years, and 2 y/o females do not reproduce when their body size is lower than a threshold. The litter size increases under a quadratic function with an improvement at the youngest ages. Interestingly, the slope of the classical positive relationship between litter size and body size in reptiles (Massot *et al.* 1992) is weaker in young than in old females (Fig. 3a). This indicates a suboptimal fecundity for a given body size in young females, and thus provides additional evidence for the reproductive maturation early in adulthood. Higher performances with age are also present late in adulthood for five variables (body size and corpulence in offspring males and females, litter success in females with a high investment in their first reproduction). All these variables are related to offspring quality. This high reproductive performance in late adulthood provides evidence for a termi-

nal investment pattern (Stearns 1992; Descamps *et al.* 2007). The terminal investment scenario is expected with senescence, i.e. with a decline of the reproductive value in old individuals (Bowen *et al.* 2006; Nussey *et al.* 2008; Reed *et al.* 2008). We showed such a senescent pattern for survival probability and litter success. The survival decline with age appeared specific to females that invested more in their first reproduction, and thus is evidence for a delayed reproductive cost. This reproductive cost supports the disposable soma and antagonistic pleiotropy theories (Gustafsson & Part 1990; Nussey *et al.* 2006) while no effect of early reproductive performance is expected on age-specific accumulation of deleterious mutations (Medawar 1952). This is a key finding because the understanding of senescent patterns is limited by the rarity of empirical evidences in wild populations. Therefore, our result reinforces the very few studies that tested and showed this reproductive cause of senescence (Gustafsson & Part 1990; Orell & Belda 2002; Nussey *et al.* 2006). In particular, this shows for the first time the trade-off between early reproductive performance and longevity in a reptile. Moreover, we found that the decline in adult female survival starts 2 years after the first reproduction (Fig. 6a; the best selected models were for the quadratic decline and threshold effect after the age of 4 years) while fecundity increases until the age of 4 years (Fig. 3a). This pattern is in accordance with the hypothesis that senescence may be delayed in species where adults continue to increase their fecundity (Vaupel *et al.* 2004).

PROXIMATE FACTORS OF AGE VARIATION

Among the various physiological measurements that might be involved in age variation (Patnaik 1994; Nemoto & Finkel 2004; Robert & Bronikowski 2010), we focused on endocrinology, metabolism and immunity, three key aspects of physiology (Ricklefs & Wikelski 2002). Among the few studies investigating ageing variation of metabolic rate, decline with age and lack of age variation are the most frequent observations (Speakman 2005; Moe *et al.* 2009). In reptiles, evidence was found for a decline early in life (Patnaik 1994). Thus, the elevation of metabolic rate we observed late in life is surprising. As we found this elevation only in 1 year, further data on additional years is needed to validate this result. If true, this elevation remains difficult to interpret because it may either reflect a physiological disruption or a good functionality (Wickens 1998). It could result from the deterioration of the hypothalamo-pituitary-adrenal axis associated with corticosterone elevation as observed in old rats and humans (Sapolsky, Krey & McEwen 1986). However, we did not find age variation in the baseline corticosterone level. The metabolic elevation could on the other hand be linked to the increase of the immune response we found, and reflect a higher allocation of resources for the body maintenance in older females.

Although some evidence for immunosenescence has been found in some free-living species, this degeneration of the immune system in old age is still unclear (Palacios *et al.* 2007). Because individuals with high T cell-mediated responses are usually considered of a better quality on the short-term, the highest response to PHA in the oldest females could explain part of our terminal investment patterns. Because of the lack of individual repeated measurements, we cannot discard the possibility of an individual heterogeneity (Forslund & Part 1995; McCleery *et al.* 2008). However, the better quality of individuals with higher responses to PHA has been found in *L. vivipara* (Cote *et al.* 2010). In addition, a positive relationship between PHA response and offspring size, which also varied with age, militates for a true ageing variation. The relationship was significant in offspring females ($F_{1,39} = 4.33$ $P = 0.0441$), and marginally significant in offspring males ($F_{1,43} = 3.16$ $P = 0.0824$). This positive relationship reinforces our suggestion of a role of the immune system in the terminal investment pattern observed on offspring quality.

To identify the proximate factors of senescence, we need further measurements such as deteriorations of physiological functions and DNA damage. Indeed, two main physiological theories of senescence are related to the 'oxidative stress' causing accumulation of cellular damages, and to the 'telomere attrition' causing the loss of DNA replication (Nemoto & Finkel 2004). These two physiological sources of ageing decline are under study. Because it can mediate physiological effects on fitness, behaviour is also an important aspect to consider in ageing variation (Catry *et al.* 2006; Kim *et al.* 2007; Lecomte *et al.* 2010). This is especially true in *L. vivipara* that shows age variation in behaviours related to mating strategy (Richard *et al.* 2005), mate choice (Richard *et al.*

2009) and offspring dispersal (Ronce, Clobert & Massot 1998).

VARIABILITY OF AGE RESPONSES

Little is known on environmental and individual heterogeneities of age variations, and this is a major gap in our understanding of ageing processes in natural populations (Wilson, Charmantier & Hadfield 2008). Indeed, it could be misleading to consider only average age-specific patterns because age variations may depend on environmental conditions, life-history traits and reproductive costs (Nussey *et al.* 2006; Monaghan *et al.* 2008; Reed *et al.* 2008). This is well illustrated by our findings on these three sources of heterogeneity. For environmental conditions, we found that habitat influences the relationship between maternal age and offspring male body size, and yearly conditions affect age variation in metabolism as well as the access to maturity. We also showed several influences of a key life-history trait. Indeed, body size mediates age dependence on the access to maturity, litter size, and offspring female body size. Sexual dimorphism is also to note, with a sharper decrease in survival rates with age in adult males. Another source of variability in age-specific responses we found is because of the cost of early reproduction in life. Only females with a high reproductive effort at first reproduction were subject to the actuarial senescence. This result strengthens the few studies of wild populations that investigated variation in senescence related to reproductive costs (Bonduriansky & Brassil 2005; Descamps *et al.* 2006; Nussey *et al.* 2006; Reed *et al.* 2008), and this reinforces evidence in favour of the disposable soma and antagonistic pleiotropy theories of senescence (Nussey *et al.* 2006). An interesting novelty is that the survival decrease in females with a high reproductive effort is associated with a rise in litter success and offspring quality. This might be because of a terminal investment pattern. However, we cannot discard the possibility that the high mortality of females with a high reproductive effort induces a selection in favour of females in the best condition. Further study is necessary to address the two hypotheses.

THE VIRTUES OF INTEGRATIVE STUDIES

Our study of ageing on multiple factors provided valuable advantages. First, ageing may be because of multiple non-exclusive processes (Forslund & Part 1995; Catry *et al.* 2006; McCleery *et al.* 2008) that are difficult to identify in studies on few variables. Here, we obtained evidence for maturation in young adult females, and also for both senescence and terminal investment in spite of their antagonistic effects. Secondly, it is interesting to test whether ageing responses differ between different types of traits (Bronikowski & Promislow 2005; Nussey *et al.* 2008; Lecomte *et al.* 2010). Here, maturation preferentially involved variables linked to early physiological stages of reproduction (access to reproduction, litter size) compared to terminal investment (offspring quality). Senescent patterns were found both on survival and repro-

duction (litter success). Thirdly, the small number of old individuals in natural populations makes it difficult to perform powerful statistical tests, and thus congruent results on several variables could reinforce a line of evidence. Here, the significant difference of immune response between 2 and 4 y/o females and older females was not sufficient to be translated to a significant average age effect or quadratic age variation. We obtained more confidence in this response because our immune measurement was related to offspring quality that also increased with age. Fourthly, the form of senescence may either be progressive or only affect old individuals (Catry *et al.* 2006). This point is still in debate (Nussey *et al.* 2008) partly because of the low statistical power of tests comparing different forms based on a single distribution/variable. Here, our overall analysis on female survival rates could not discriminate between linear, quadratic and threshold effects. The additional analysis based on the reproductive effort at the first reproduction allowed us to show the form of senescence as a nonlinear decrease of survival with age (quadratic or threshold effect after age of 4 years). This nonlinear age variation late in adulthood also characterized the immune response. Finally, investigating physiology is especially relevant because most ageing variations should have a physiological basis. Here, we found age variations in metabolic rate and immunity, and the immune response is related to offspring body size in the context of a terminal investment pattern. All these points clearly show the interest to develop integrative approaches to improve our knowledge of the multifaceted processes of ageing acting in free-living populations.

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References

- Andrews, R.M. & Pough, F.H. (1985) Metabolism of squamate reptiles – allometric and ecological relationships. *Physiological Zoology*, **58**, 214–231.
- Bonduriansky, R. & Brassil, C.E. (2005) Reproductive ageing and sexual selection on male body size in a wild population of antler flies (*Protopiophilila litigata*). *Journal of Evolutionary Biology*, **18**, 1332–1340.
- Bowen, W.D., Iverson, S.J., McMillan, J.I. & Boness, D.J. (2006) Reproductive performance in grey seals: age-related improvement and senescence in a capital breeder. *Journal of Animal Ecology*, **75**, 1340–1351.
- Bronikowski, A.M. & Promislow, D.E.L. (2005) Testing evolutionary theories of aging in wild populations. *Trends in Ecology & Evolution*, **20**, 271–273.
- Catry, P., Phillips, R.A., Phalan, B. & Croxall, J.P. (2006) Senescence effects in an extremely long-lived bird: the grey-headed albatross *Thalassarche chrysoloma*. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 1625–1630.
- Clobert, J., Lebreton, J.D. & Allaine, D. (1987) A general approach to survival rate estimation by recaptures or resightings of marked birds. *Ardea*, **75**, 133–142.
- Clobert, J., Massot, M., Lecomte, J., Sorci, G., De Fraipont, M. & Barbault, R. (1994) Determinants of dispersal behavior: the common lizard as a case study. *Lizard Ecology: Historical and Experimental Perspectives* (eds L.J. Vitt & R. Pianka), pp. 183–206. Princeton University Press, Princeton.
- Congdon, J.D., Nagle, R.D., Kinney, O.M. & Sels, R.C.V. (2003) Testing hypotheses of aging in long-lived painted turtles (*Chrysemys picta*). *Experimental Gerontology*, **38**, 765–772.
- Cote, J., Meylan, S., Clobert, J. & Voituren, Y. (2010) Carotenoid-based coloration, oxidative stress and corticosterone in common lizards. *Journal of Experimental Biology*, **213**, 2116–2124.
- Dauphin-Villemant, C. & Xavier, F. (1987) Nychthemeral variations of plasma corticosteroids in captive female *Lacerta vivipara* Jacquin: influence of stress and reproductive state. *General and Comparative Endocrinology*, **67**, 292–302.
- Descamps, S., Boutin, S., Berteaux, D. & Gaillard, J.M. (2006) Best squirrels trade a long life for an early reproduction. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 2369–2374.
- Descamps, S., Boutin, S., Berteaux, D. & Gaillard, J.M. (2007) Female red squirrels fit Williams' hypothesis of increasing reproductive effort with increasing age. *Journal of Animal Ecology*, **76**, 1192–1201.
- Fitze, P.S., Cote, J., San-Jose, L.M., Meylan, S., Isaksson, C., Andersson, S., Rossi, J.-M. & Clobert, J. (2009) Carotenoid-based colours reflect the stress response in the common lizard. *PLoS ONE*, **4**, e5111.
- Forslund, P. & Part, T. (1995) Age and reproduction in birds – hypotheses and tests. *Trends in Ecology & Evolution*, **10**, 374–378.
- Gustafsson, L. & Part, T. (1990) Acceleration of senescence in the collared flycatcher *Ficedula albicollis* by reproductive costs. *Nature*, **347**, 279–281.
- Hamilton, W.D. (1966) Moulding of senescence by natural selection. *Journal of Theoretical Biology*, **12**, 12–45.
- Jones, O.R., Gaillard, J.M., Tuljapurkar, S., Alho, J.S., Armitage, K.B., Becker, P.H., Bize, P., Brommer, J., Charmantier, A., Charpentier, M., Clutton-Brock, T., Dobson, F.S., Festa-Bianchet, M., Gustafsson, L., Jensen, H., Jones, C.G., Lilland, B.G., McCleery, R., Merila, J., Neuhaus, P., Nicoll, M.A.C., Norris, K., Oli, M.K., Pemberton, J., Pietiainen, H., Ringsby, T.H., Roulin, A., Saether, B.E., Setchell, J.M., Sheldon, B.C., Thompson, P.M., Weimerskirch, H., Wickings, E.J. & Coulson, T. (2008) Senescence rates are determined by ranking on the fast-slow life-history continuum. *Ecology Letters*, **11**, 664–673.
- Kim, S.Y., Torres, R., Rodriguez, C. & Drummond, H. (2007) Effects of breeding success, mate fidelity and senescence on breeding dispersal of male and female blue-footed boobies. *Journal of Animal Ecology*, **76**, 471–479.
- Kirkwood, T.B.L. (1977) Evolution of aging. *Nature*, **270**, 301–304.
- Lecomte, V.J., Sorci, G., Cornet, S., Jaeger, A., Faivre, B., Arnoux, E., Gaillard, M., Trouve, C., Besson, D., Chastel, O. & Weimerskirch, H. (2010) Patterns of aging in the long-lived wandering albatross. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 6370–6375.
- Low, M., Part, T. & Forslund, P. (2007) Age-specific variation in reproduction is largely explained by the timing of territory establishment in the New Zealand stitchbird *Notiomystis cincta*. *Journal of Animal Ecology*, **76**, 459–470.
- Martin, L.B., Scheuerlein, A. & Wikelski, M. (2003) Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 153–158.
- Massot, M. & Clobert, J. (2000) Processes at the origin of similarities in dispersal behaviour among siblings. *Journal of Evolutionary Biology*, **13**, 707–719.
- 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.
- McCleery, R.H., Perrins, C.M., Sheldon, B.C. & Charmantier, A. (2008) Age-specific reproduction in a long-lived species: the combined effects of senescence and individual quality. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 963–970.
- Medawar, P.B. (1952) *An Unsolved Problem of Biology*. Lewis, H. K., London.
- Meylan, S., Dufty, A.M. & Clobert, J. (2003) The effect of transdermal corticosterone application on plasma corticosterone levels in pregnant *Lacerta vivipara*. *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*, **134**, 497–503.
- Meylan, S., Haussy, C. & Voituren, Y. (2010) Physiological actions of corticosterone and its modulation by an immune challenge in reptiles. *General and Comparative Endocrinology*, **169**, 158–166.
- Moe, B., Ronning, B., Verhuist, S. & Bech, C. (2009) Metabolic ageing in individual zebra finches. *Biology Letters*, **5**, 86–89.
- Monaghan, P., Charmantier, A., Nussey, D.H. & Ricklefs, R.E. (2008) The evolutionary ecology of senescence. *Functional Ecology*, **22**, 371–378.

- Nemoto, S. & Finkel, T. (2004) Ageing and the mystery at Arles. *Nature*, **429**, 149–152.
- Norris, K. & Evans, M.R. (2000) Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology*, **11**, 19–26.
- Nussey, D.H., Kruuk, L.E.B., Donald, A., Fowlie, M. & Clutton-Brock, T.H. (2006) The rate of senescence in maternal performance increases with early-life fecundity in red deer. *Ecology Letters*, **9**, 1342–1350.
- Nussey, D.H., Coulson, T., Festa-Bianchet, M. & Gaillard, J.M. (2008) Measuring senescence in wild animal populations: towards a longitudinal approach. *Functional Ecology*, **22**, 393–406.
- Orell, M. & Belda, E.J. (2002) Delayed cost of reproduction and senescence in the willow tit *Parus montanus*. *Journal of Animal Ecology*, **71**, 55–64.
- Palacios, M.G., Cunnick, J.E., Winkler, D.W. & Vleck, C.M. (2007) Immunosenescence in some but not all immune components in a free-living vertebrate, the tree swallow. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 951–957.
- Patnaik, B.K. (1994) Ageing in reptiles. *Gerontology*, **40**, 200–220.
- Reed, T.E., Kruuk, L.E.B., Wanless, S., Frederiksen, M., Cunningham, E.J.A. & Harris, M.P. (2008) Reproductive senescence in a long-lived seabird: rates of decline in late-life performance are associated with varying costs of early reproduction. *American Naturalist*, **171**, E89–E101.
- Richard, M., Lecomte, J., De Fraipont, M. & Clobert, J. (2005) Age-specific mating strategies and reproductive senescence. *Molecular Ecology*, **14**, 3147–3155.
- Richard, M., Losdat, S., Lecomte, J., De Fraipont, M. & Clobert, J. (2009) Optimal level of inbreeding in the common lizard. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 2779–2786.
- Ricklefs, R.E. & Wikelski, M. (2002) The physiology/life-history nexus. *Trends in Ecology & Evolution*, **17**, 462–468.
- Robert, K.A. & Bronikowski, A.M. (2010) Evolution of senescence in nature: physiological evolution in populations of garter snake with divergent life histories. *American Naturalist*, **175**, E47–E159.
- Ronce, O., Clobert, J. & Massot, M. (1998) Natal dispersal and senescence. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 600–605.
- Sapolsky, R.M., Krey, L.C. & McEwen, B.S. (1986) The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocrine Reviews*, **7**, 284–301.
- Sparkman, A.M., Arnold, S.J. & Bronikowski, A.M. (2007) An empirical test of evolutionary theories for reproductive senescence and reproductive effort in the garter snake *Thamnophis elegans*. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 943–950.
- Speakman, J.R. (2005) Body size, energy metabolism and lifespan. *The Journal of Experimental Biology*, **208**, 1717–1730.
- Stearns, S.C. (ed.) (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Surget-Groba, Y., Heulin, B., Guillaume, G.P., Thorpe, R.S., Kupriyanova, L., Vogrin, N., Maslak, R., Mazzotti, S., Venczel, M., Ghira, I., Odierna, G., Leontyeva, O., Monney, J.C. & Smith, N. (2001) Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. *Molecular Phylogenetics and Evolution*, **18**, 449–459.
- Vaupel, J.W., Baudisch, A., Dolling, M., Roach, D.A. & Gampe, J. (2004) The case for negative senescence. *Theoretical Population Biology*, **65**, 339–351.
- White, G.C. & Burnham, K.P. (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**, 120–139.
- Wickens, A.P. (1998) *The Causes of Ageing*. Harwood Academic Publishers, Amsterdam.
- Williams, G.C. (1957) Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, **11**, 398–411.
- Wilson, A.J., Charmantier, A. & Hadfield, J.D. (2008) Evolutionary genetics of ageing in the wild: empirical patterns and future perspectives. *Functional Ecology*, **22**, 431–442.

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Supporting Information

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Table S1. Age-specific estimates of the circulating corticosterone level.

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