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Female red squirrels fit Williams' hypothesis of increasing reproductive effort with increasing age

SÉBASTIEN DESCAMPS*†, STAN BOUTIN‡, DOMINIQUE BERTEAUX* and JEAN-MICHEL GAILLARD†

**Chaire de Recherche du Canada en Conservation des Écosystèmes Nordiques et Centre d'Études Nordiques, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, Québec G5L3A1, Canada; †Unité Mixte de Recherche 5558 'Biométrie et Biologie Évolutive', Université Claude Bernard Lyon I, 43 boul. du 11 Novembre 1918, 69622 Villeurbanne Cedex, France; and ‡Department of Biological Sciences, CW405, Biological Sciences Centre, University of Alberta, Edmonton, Alberta, T6G2E9, Canada*

Summary

1. Williams predicted that reproductive effort should increase as individuals age and their reproductive value declines. This simple prediction has proven difficult to test because conventional measures of energy expenditure on reproduction may not be a true reflection of reproductive effort.

2. We investigated age-specific variation in female reproductive effort in a stable population of North American red squirrels where energy expenditure on reproduction is likely to reflect actual reproductive effort. We used seven measures of reproductive effort spanning conception to offspring weaning.

3. We found that females completed growth by age 3 and that reproductive value decreased after this age likely because of reproductive and survival senescence. We therefore, predicted that reproductive effort would increase from age 3 onwards.

4. The probability of breeding, litter mass at weaning, and likelihood of territory bequeathal were all lower for 1- and 2-year-old females than for females older than 3 years, the age at which growth is completed. That growing females are faced with additional energetic requirements might account for their lower allocation to reproduction as compared with older females.

5. The probability of attempting a second reproduction within the same breeding season and the propensity to bequeath the territory to juveniles increased from 3 years of age onwards, indicating an increase in reproductive effort with age. We think this increase in reproductive effort is an adaptive response of females to declining reproductive values when ageing, thereby supporting Williams' prediction.

Key-words: allocation, general life-history problem, investment, reproductive value, *Tamiasciurus hudsonicus*.

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Introduction

One central question in evolutionary ecology is to understand how individuals optimize resource allocation to their growth, survival and reproduction (Stearns 1992). Schaffer (1983) refers to this question as the 'general life-history problem'. Williams (1966) predicted that reproductive effort, as measured by the 'fraction of available energy devoted to reproduction' (Charlesworth & Leòn 1976), should increase from the first breeding attempt to the end of life in the typical case where residual reproductive value decreases with age (see – Schaffer 1974; Charlesworth & Leòn 1976 – for demonstrations of Williams' hypothesis). Indeed, as the chance of surviving and breeding before dying decreases when ageing, individuals should increase their current reproductive effort. This has led to the concept of terminal investment (e.g. Clutton-Brock

Correspondence: Chaire de Recherche du Canada en Conservation des Écosystèmes Nordiques, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, Québec G5L 3A1, Canada. Tel.: 1 418 723 1986, ext. 1909. Fax: 1 418 724 1849. E-mail: dominique_berteaux@uqar.qc.ca

1984), although increased investment (*sensu* Trivers 1972) can occur for a given or even decreasing reproductive effort. Despite its intuitive attractiveness, Williams' hypothesis only holds under particular conditions (e.g. Fagen 1972; Charlesworth & Leòn 1976; Michod 1979), in particular, when populations are stable (Charlesworth & Leòn 1976; Roff 2002, pp. 230–236 for a synthesis).

Tests of Williams' hypothesis have proven challenging because measurement of actual reproductive effort requires knowing both the total energy available to an individual as well as the total amount expended in reproduction (Clutton-Brock 1984). Measures of energy expenditure on reproduction may not be a true reflection of reproductive effort when total energy available to an individual varies with age (young and old individuals may be socially subordinate to prime-aged individuals or be less efficient at processing resources because of experience or senescence).

Furthermore, as low-quality individuals are expected to die first (Service 2000), the average quality of individuals is expected to increase with age (Cam *et al.* 2002) and this within-cohort selection leads to an increase in the proportion of high-quality individuals among old age classes. Any apparent increase in measures of reproductive effort could then be due to the above-average quality of old individuals at the population level and not to an increase in effort at the individual level. This problem can be circumvented by the use of individual longitudinal data (individuals are monitored over their lifetime) and the use of appropriate statistical methods (e.g. mixed models, Cam *et al.* 2002; Steele & Hogg 2003; van de Pol & Verhulst 2006). Lastly, age variation in effort may be expressed in some but not all components of parental care. For example, individuals can gain experience as they age, and may compensate for a lower condition by adopting a wiser 'energetic input strategy' (Cameron *et al.* 2000) by investing only at some key periods of the cycle (e.g. when the response of offspring survival to an extra allocation is the highest; Clutton-Brock 1984). In addition, trade-offs between components of maternal care may occur, so that measuring only one component of reproductive effort might provide a biased picture of the total reproductive effort. A full assessment of all components of reproductive effort spanning over the entire parental care period is therefore necessary to assess age-specific variation in reproductive effort reliably.

Given the above, it is difficult to estimate age-specific variation in reproductive effort in the field but long-term studies of individuals provide a way to circumvent many of the potential problems. Parental care of most mammals is limited to females and corresponds to a two-stage process combining viviparity and lactation. This engages the full metabolic machinery of females during reproduction, as most of the energy for offspring growth is first processed by mothers. Female mammals thus offer critical tests for evolutionary theories dealing with reproductive effort. We investigated age-specific reproductive effort in female American red squirrels *Tamiasciurus hudsonicus* studied over a 16-year period at Kluane, Yukon territory, Canada (Berteaux & Boutin 2000; Humphries & Boutin 2000; Boutin *et al.* 2006). American red squirrels have a number of advantages for the study of age-related reproductive effort, the most significant being that access to resources does not change systematically as a function of an individual's age. Individual red squirrels defend food-based territories year round and these territories are acquired in the autumn of the year of birth. Territory size does not change after 1 year of age (Lamontagne 2007) and squirrels rarely change territory location. The only exception is when adult females bequeath their territory to one of their offspring (Price & Boutin 1993; Berteaux & Boutin 2000). In these instances, females leave their territory to their offspring and settle on an unoccupied territory nearby but there is no evidence that bequeathing females move to higher quality territories (Larsen & Boutin 1995; Berteaux & Boutin 2000).

Secondly, because we were able to measure the weights of individual females regularly and follow offspring from birth to independence, we were able to get a complete picture of reproductive effort. In addition to litter mass (at birth and at weaning) and mass changes of adults during reproduction, which are commonly used to reflect energy allocation during reproduction (e.g. Michener & Locklear 1990; Kojola 1991; Bowen *et al.* 1994; Millesi *et al.* 1999; Ericsson *et al.* 2001; Weladji *et al.* 2002; Yoccoz *et al.* 2002; Broussard *et al.* 2005; Mysterud, Solberg & Yoccoz 2005), we also recorded the propensity to breed each year as well as the propensity to attempt a second reproduction within the same season. These two variables reflect substantial energy input into reproduction. Indeed, mating chases in promiscuous red squirrels (Smith 1968; Boutin & Larsen 1993) occur at the end of winter (from February to April), when temperatures are still low. Thus, energy expended to find a mate and reproduce is likely to be high. We also measured the propensity for females to bequeath their territory to their young at weaning (Price & Boutin 1993; Berteaux & Boutin 2000; Boutin, Larsen & Berteaux 2000). This behaviour can be interpreted as the final form of allocation by females before juveniles are completely independent.

The goal of our study was to assess age variation in female reproductive effort in this North American red squirrel population. We made the following two a priori predictions: first, as female squirrels grow until 3 years of age (Descamps 2006), we expected reproductive effort to be lower for 1- and 2-year-old females than for older females because of the additional energy requirements of growth (Stearns 1992). Second, females show both decreased survival and probability of breeding successfully after 3 years of age (Descamps 2006; McAdam *et al.*, in press), which leads to a decrease in reproductive values from 3 years of age onwards (see

Materials and methods). Given that yearly population growth rate was close to zero during the study period ($r = 0{\cdot}009$, McAdam *et al.*, in press) we predicted after Williams (1966) and Charlesworth & Leòn (1976) that reproductive effort of females should increase from 3 years of age onwards.

Materials and methods

STUDY AREA AND RED SQUIRREL POPULATION

Our work was conducted in the South Yukon, Canada (61°N, 138°W) and we report results for the years 1988–2003 for two 40 ha control grids separated by the Alaska Highway. The squirrel populations on these sites averaged population growth rates near zero over the course of the study (McAdam *et al.*, in press). Habitat is boreal forest with white spruce *Picea glauca* as the dominant tree species. Spruce seeds represent the main food resource for red squirrels in this population (McAdam & Boutin 2003; Boutin *et al.* 2006). At the end of summer, red squirrels store unopened cones in central hoarding sites called middens (Humphries *et al.* 2005). All squirrels were marked with numbered ear tags and a unique colour combination of wires for identification at a distance. Fates of female squirrels were determined through live-trapping (Tomahawk traps) and visual observations from April to August. The breeding status of females was determined through nipple condition, changes in mass and/or lactation. The nest of each breeding female was visited soon after birth to count, weigh and sex the young. Juveniles were tagged and weighed at about 20–25 days of age and weighed within a week of weaning (about 70 days of age). Females were weighed soon after parturition and again within a week of weaning their offspring. Females were also trapped and weighed regularly throughout the breeding season, which allowed us to weigh females around the time of emergence of their offspring (i.e. time when juveniles begin to leave the nest, between 35 and 45 days after parturition, Humphries & Boutin 1996). Weights were determined to the nearest gram with a Pesola scale.

AGE-SPECIFIC VARIATION IN REPRODUCTIVE VALUES

Previous studies in the same red squirrel population (Descamps 2006; McAdam *et al.*, in press) reported that both survival and breeding success decrease from 3 years of age onwards (Fig. 1 inset). We used the Leslie matrix model to calculate age-specific reproductive values. As reviewed by Caswell (2001), the dominant eigenvalue (λ) of the matrix gives the population growth rate. The associated right and left eigenvectors w and v give the stable age distribution and the age-specific reproductive values, respectively. We thus calculated the age-reproductive values as the left eigenvector of the population projection matrix including age-specific estimates of breeding success (first row) and of survival (subdiagonal) displayed in the insert of Fig. 1.

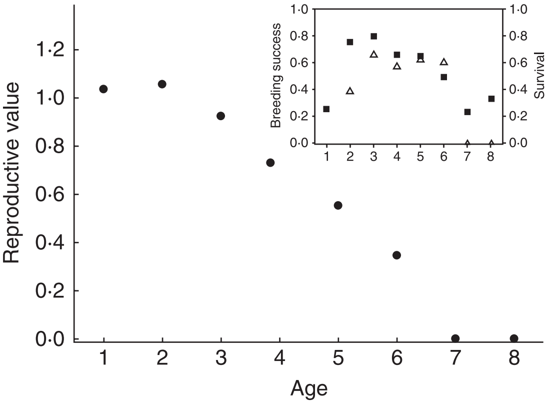

Fig. 1. Age-specific reproductive values of females in a population of North American red squirrels, studied at Kluane, Yukon, Canada. Inset shows survival rates (black squares; from McAdam *et al.*, in press) and breeding success (white triangles; estimated as the product of litter size at birth, proportion breeding, and offspring survival from birth to weaning) at a given age.

STUDY DESIGN

We assessed the influence of age on female reproductive effort using a suite of dependent variables reflecting the diversity of physiological and behavioural processes involved in squirrel reproduction. Our choice of measures of reproductive effort components thus encompassed key parameters hereafter organized chronologically from the propensity to breed to the weaning of offspring.

We first investigated the effect of mother's age on breeding propensity (i.e. the probability to breed or not). We considered that a female was breeding when she gave birth to at least one juvenile. Mass of females before reproduction may reflect females' condition and thus affect the probability to breed. For a subset of females ($n = 80$), mass before the onset of reproduction (i.e. February) was known. We thus studied age-specific variation in breeding probability for the whole set of females without adjusting for their mass, and also for the subset of females whose mass was known including mass as a covariate in our models. In 1993 and 1998, the two mast years (i.e. highest cone production, McAdam *et al.*, in press), some females that successfully weaned offspring at their first breeding attempt bred a second time within the same breeding season (Boutin *et al.* 2006). We thus investigated the effect of mother's age on the probability to breed twice within a year.

Humphries & Boutin (1996) suggested that in this red squirrel population, females adjust the energy stored during the early lactation period (from parturition to emergence) to the future reproductive demands of late lactation. As the energy stored during early lactation is used during late lactation (from emergence to weaning), mass loss during the late lactation period should be a relevant indicator of mother's energy allocation to reproduction. We thus considered female mass change between parturition and weaning (a measure of mother's reproductive effort during the whole lactation) as well as female mass change between the emergence of offspring and weaning (a measure of mother's reproductive effort during late lactation). We performed analyses by adjusting (i.e. assessing variation in the energy allocated per juvenile) or not (i.e. assessing variation in the energy allocated per litter) for litter size.

In a third step, we studied the effects of mother's age on litter mass at parturition and at weaning. As we did not get mass at weaning for all weaned juveniles, mass of a given litter was measured as the product of the number of weaned juveniles from a given litter times the average mass of juveniles from this litter for which weaning mass was known. To analyse age-specific variation in litter mass (both at birth and at weaning), we included the mass of the mother after parturition in our models because mass varies in relation to age in this red squirrel population (Descamps 2006). For litter mass at birth, we considered litters weighed within 25 days after parturition (as growth is linear during this period, McAdam & Boutin 2003), and we included in our models the age at which juveniles were weighed. Finally, we investigated the relationship between female's age and the probability to bequeath the territory at weaning (Price & Boutin 1993; Berteaux & Boutin 2000). Territory bequeathal was measured as a binary variable: the female keeps her territory vs. gives part or all of her territory to one or several of her juveniles. Berteaux & Boutin (2000) showed that females bequeathing their territory are older on average than females keeping it. However, it was unclear whether this age effect was simply due to a difference between 1-year-old and older mothers, or whether it resulted from a more continuous effect of age on the probability to bequeath their territory.

STATISTICAL ANALYSES

Data from the two study grids were pooled as including 'grid' as a factor in our analyses did not affect results. To assess the relationship between age and reproductive effort (expected to be nonlinear), we used Generalized Additive Models (Hastie & Tibshirani 1990). Such models assume that the dependent variable depends on additive predictors that are fitted using spline-smoothers. The optimal complexity of the splines (i.e. degrees of freedom) were determined using the GCV (generalized cross-validation) option in procedure GAM, SAS version 9 (SAS Institute 2004). We built GAM that modelled the expected value of the dependent variable Y (i.e. measure of effort) as: $E(Y) = a + b.Year + S(Age)$, where S is the smooth function of interest in this study. We included a variable *Year* as a discrete factor to account for between-year variation in environmental conditions.

Our data set included repeated measurements of the same individuals at different ages (average of 1·2–2·4 measures per individual according to the trait considered; mode of one measure per individual for all traits, range = [1, 7]). We thus fitted in a second step generalized linear mixed models (GLMM) with individual identity included as a random effect (with the MIXED procedure and the GLIMMIX macro of SAS, Littell *et al.* 1996) to avoid pseudo-replication problems (Hurlbert 1984). To characterize the functional relationship between reproductive effort and age, we compared different continuous and discrete models that described different patterns of age-specific variation in reproductive effort (model selection was based on AIC, Burnham & Anderson 2002). Including identity as a random factor in mixed models did not increase the fit of the models, and using GLM led to the same results as GLMM. For continuous dependent variables, normality and homogeneity of residuals have been assessed using Shapiro–Wilk tests and graphical inspection of residuals plotted against fitted values, and for binary variables, goodness-of-fit of the logistic regression models were assessed using Hosmer–Lemeshow tests (all P-values were $> 0{\cdot}18$). This indicates that, despite some pseudo-replication in our data, standard analyses not adjusted for repeated measurements provided reliable parameter estimates. As the use of linear models (both with or without random effects) led to the same results as GAM, we only presented results from GAM.

Proportion of variance explained by a given model was calculated as coefficient of determination (R^2) for analyses of mass change and litter mass and as max-rescaled R^2 (Nagelkerke 1991) for analyses of breeding and bequeathal probabilities.

Results

The effect of year was significant in all analyses, indicating that reproductive effort changed according to environmental conditions. Although yearly variations in reproductive effort can be partly accounted for by cone availability (results not shown), we did not look here at specific relationships between food availability and reproductive effort, and we simply included the factor year in all models.

BREEDING PROPENSITY

Breeding propensity increased from about 50% at 1 year of age to 100% at 4 years of age, and remained constant thereafter (Fig. 2a). GAM indicated a significant positive linear effect of age on a logit scale ($n = 782$ observations performed on 322 females; slope of 1·55; 95% confidence interval = [1·25; 1·90]; $t = 10{\cdot}81$, $P < 0{\cdot}001$; see inset in Fig. 2a). The smoothing component of the age effect was not significant ($P = 0{\cdot}12$), so that a linear model provided an adequate fit of age variation in breeding probabilities. Thus, a simple

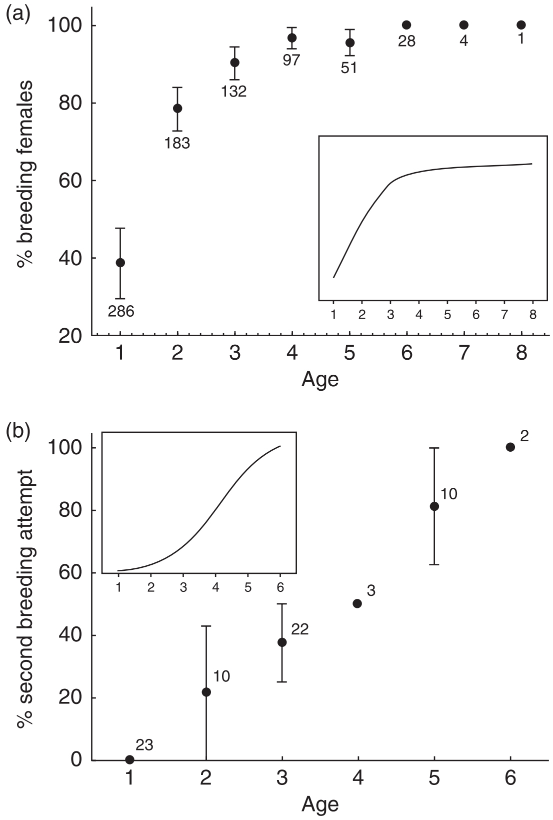

Fig. 2. Age and breeding propensity in a population of North American red squirrels, studied at Kluane, Yukon, Canada (mean ± SE). (a) Proportion of reproductive females in relation to age (mean ± SE). (b) Proportion of second reproduction attempts according to age in 2 years when a second reproduction was observed within the same breeding season (1993 and 1998; mean ± SE). Inset in both plots represent the predicted effect of age on breeding probabilities (from a model adjusted for yearly variations). Numbers correspond to age-specific sample sizes.

linear model including age and year (as a categorical factor) accounted for 47% of observed variation in breeding probability. When considering the subset of females for which mass before reproduction was known (*n* = 80), the effect of mass was not significant (*t* = 0·27, *P* = 0·78).

In the two mast years, 27% (19 of 70) of females that successfully weaned ≥ 1 offspring attempted to breed again within the same reproductive season. The probability to breed a second time given a first successful reproduction increased with age from 0 at 1 year of age to 1 at 6 years of age (Fig. 2b). Such an increase was statistically significant (*n* = 70 observations performed on 69 females; slope of 1·26; 95% confidence interval = [0·70; 2·00] on a logit scale; *t* = 3·83, *P* < 0·001; see inset in Fig. 2b) and the smoothing effect of age was not significant (*P* = 0·49), indicating that the probability to breed twice within the same season increased linearly (on a logit scale) with increasing age. A linear model including year and age accounted for 51% of the observed variation in the probability to breed twice within a season.

FEMALE MASS CHANGE DURING REPRODUCTION

The average proportion of mass change between parturition and weaning of juveniles was –0·013 ± 0·005 SE (*n* = 265 measurements performed on 165 females), which corresponds to an average mass loss of 5 g (Fig. 3a). GAM indicated that the linear component of the age effect was negative but not significant (slope of –0·005; 95% confidence interval = [–0·011; 0·001]; *t* = –1·63, *P* = 0·10), and that no smoothing effect was required to explain age variation in mass change (*P* > 0·99).

The average proportion of mass change between emergence and weaning of juveniles was –0·037 ± 0·004 SE (*n* = 265 measurements performed on 165 females), which corresponds to an average mass loss of 10 g (Fig. 3b) and did not vary with age. GAM indicated that neither the linear component of the age effect (slope of 0·0004; 95% confidence interval = [–0·005; 0·006]; *t* = 0·17, *P* = 0·87) nor its smoothing component (*P* > 0·99) were significant.

Results remained the same when we considered absolute change in mass or change in mass relative to litter size, and litter size did not influence the

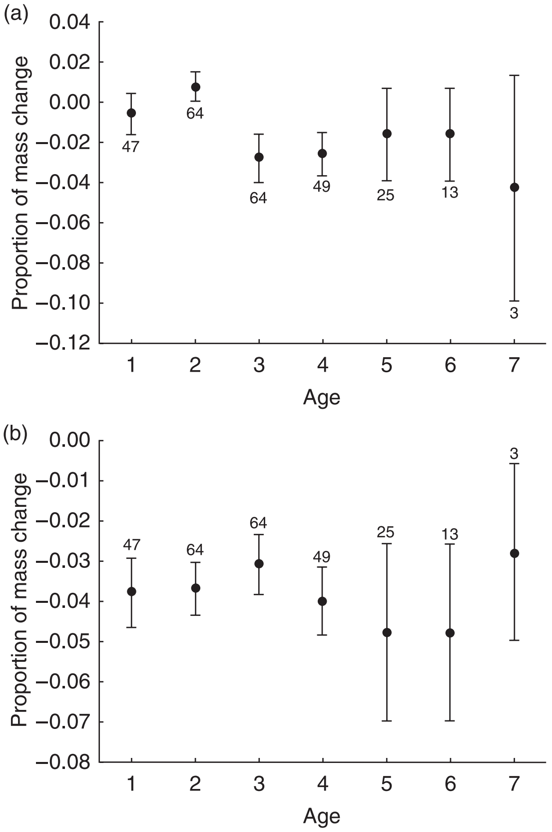

Fig. 3. Age of and proportion of mass change in reproductive females in a population of North American red squirrels, studied at Kluane, Yukon, Canada (mean ± SE). (a) Mass change between parturition and offspring weaning, (b) mass change between emergence and offspring weaning. Numbers correspond to age-specific sample sizes.

proportion of mass lost (results associated to the litter size effect for mass loss between parturition and weaning, and between emergence and weaning, respectively: $t = 0{\cdot}04$, $P = 0{\cdot}97$ and $t = 1{\cdot}62$, $P = 0{\cdot}10$).

LITTER MASS

Total litter mass at birth averaged 58·9 g ± 1·9 SE (average age at which juveniles were weighed: 6·7 days ± 0·3 SE), and did not vary with age of the mother ($n = 328$ measurements performed on 203 females; slope of 0·60; 95% confidence interval = [–1·09; 2·30]; $t = 0{\cdot}70$, $P = 0{\cdot}49$; effect of the smoothing component not significant: $P > 0{\cdot}99$; Fig. 4a). The mass of the mother had a nearly significant positive effect on the mass of the litter at birth (estimate: 0·108, 95% confidence interval = [–0·005; 0·221]; $t = 1{\cdot}82$, $P = 0{\cdot}063$). This model, including a linear effect of age, the mass of the mother, the age at which juveniles were weighed and a year effect accounted for 67% of the observed variation in litter mass at birth. When including the size of the litter in this model, we found similar results indicating that the average mass of juveniles at birth did not vary as a function of mother's age.

Total litter mass at weaning averaged 299·4 g ± 10·9 SE ($n = 161$ measurements made on litters of 131 females) and was maximum at 3 years of age (total litter mass at 3 years old: 355·2 g ± 22·6 SE; Fig. 4b). The mass of the mother did not influence the total mass of the litter at weaning ($t = 0{\cdot}51$, $P = 0{\cdot}61$). GAM indicated that the linear effect of age was not significant ($t = 0{\cdot}80$, $P = 0{\cdot}43$) but the smoothing effect was (decrease in deviance when including the smoothing effect = 10·96 on 1·3 d.f., $P = 0{\cdot}0015$). GAM showed that the relationship between age and litter mass at weaning was roughly quadratic, with an increase in litter mass between 1 and 3 years of age, followed by a decrease. A quadratic linear model accounted for 21% of the observed variation in litter mass with an increase of about 100 g between 1 and 3 years of age, followed by a decrease of 90 g between 4 and 6 years of age (see inset in Fig. 4b). When adjusting for litter size at weaning (i.e. number of offspring successfully weaned), the age effect was no longer significant (linear component: $t = 0{\cdot}12$, $P = 0{\cdot}90$; smoothing component: $P = 0{\cdot}10$), indicating that variation in total litter mass at weaning was the consequence of variation in litter size at weaning.

TERRITORY BEQUEATHAL

The proportion of females bequeathing their territory to offspring at weaning averaged 52·0% ± 6·5 SE ($n = 341$ observations performed on 186 females) and increased regularly from just over 40% at age 1 to between 80 and 100% at age 6 and 7 (Fig. 5). GAM indicated that the linear component of age was significant (slope of 0·35; 95% confidence interval = [0·12; 0·60] on a logit scale; $t = 10{\cdot}81$, $P = 0{\cdot}0035$; see inset in Fig. 5) but not the smoothing one ($P > 0{\cdot}99$). A linear model including year as a categorical factor and a linear effect of age on a logit scale accounted for 42% of the observed variation in bequeathal probability.

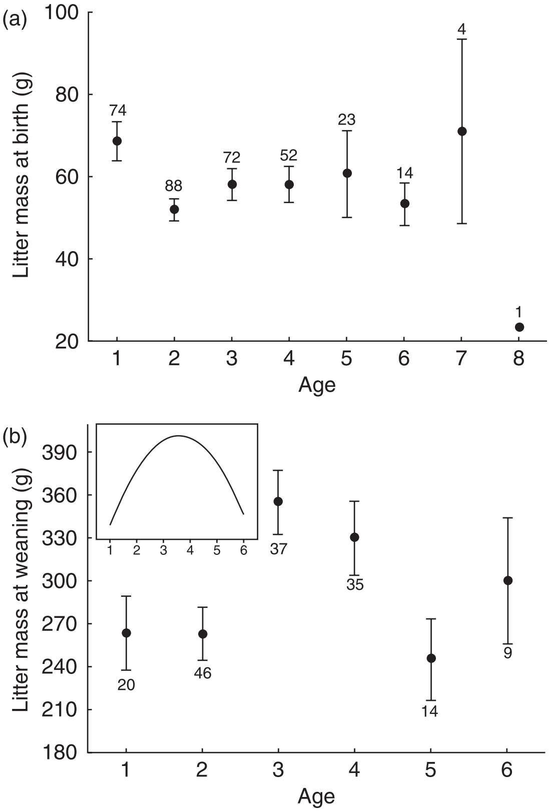

Fig. 4. Age of breeding females and litter mass in a population of North American red squirrels, studied at Kluane, Yukon, Canada (mean ± SE). (a) Litter mass at birth, (b) litter mass at weaning. Inset in figure (b) represents the predicted effect of age from a model adjusted for yearly variations and mother's mass. Numbers correspond to age-specific sample sizes.

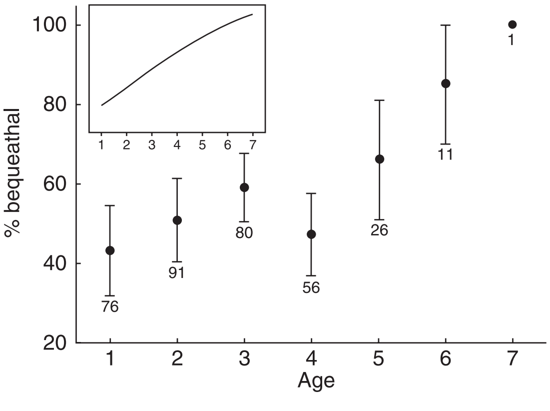

Fig. 5. Age of breeding females and probability to bequeath territory at weaning in a population of North American red squirrels, studied at Kluane, Yukon, Canada (mean ± SE). Inset shows the predicted effect of age (from a model adjusted for yearly variations). Numbers correspond to age-specific sample sizes.

Discussion

REPRODUCTIVE EFFORT AND AGE IN NORTH AMERICAN RED SQUIRRELS

We predicted that female squirrels in our study population should show increasing levels of reproductive effort as they aged but the reasons for this increase would differ depending on age. Increases to age 3 were expected because younger females had to meet both growth and reproductive demands whereas increases after age 3 were predicted because this was the age at which reproductive value declined. We found that standard mammalian measures of reproductive effort such as litter mass at birth and mass change of females during reproduction did not change in relation to age. However, litter mass at weaning increased until 3 years of age and decreased thereafter. The propensity to breed increased to age 4 and remained constant thereafter whereas the probability of producing a second litter in mast years after a first successful reproductive attempt within the same breeding season and the probability of bequeathing part of or all the territory to offspring increased steadily with age. We suggest the following explanations for these results.

Our study indicated no age variation in mass change during reproduction. American red squirrels are income breeders (*sensu* Jönsson 1997) because they rely on a food cache (i.e. spruce cones hoarded from the previous autumn) to meet energy requirements during reproduction. Resource availability (and quality) and foraging activity of females are thus likely to be more influential than mass change of females to explain the relationship between reproductive effort and age (see Tuomi, Hakala & Haukioja 1983 for a similar viewpoint). Therefore, mass change may be irrelevant to study variation in reproductive effort in populations of income breeders (see e.g. Andersen *et al*. 2000 for a case study on roe deer).

Litter mass at birth showed no relation to age, whereas litter mass at weaning was lower in young and old squirrels. Thus, despite producing a similar number of offspring, young and old females were less successful than prime-aged females at raising these young to weaning. In the case of young females, possible explanations are that they were less experienced as mothers and/or they showed reduced reproductive effort. We were unable to assess directly the role of experience but given that there was no increase in mass at weaning from age 1 to age 2 (Fig. 4b), it seems unlikely that experience is involved. Female body mass increased until 3 years of age in this population (Descamps 2006) and this is the age at which mass at weaning showed a large increase. This suggests that young female squirrels may show reduced reproductive effort because they have the added energetic demand of growth. The lower breeding probability, litter mass at weaning and bequeathal probability for 1- and 2-year-old female squirrels all suggest that young growing females allocate less energy to reproduction than fully grown ones.

The explanation for why older females weaned fewer offspring relative to prime-age females is likely very different. Our results suggest that old females allocated a similar amount of energy into their offspring as compared with prime-age females (i.e. they lost a similar mass during reproduction) but that this energy transfer was less efficient in older females (i.e. they weaned fewer juveniles). Old females were thus not able to raise as many offspring as prime-age ones for a given amount of energy allocated, which supports the existence of senescence (Rose 1991). Therefore, the observed decrease in litter mass for old females did likely not correspond to a decrease in reproductive effort, but rather represents the decreasing reproductive abilities of old females.

Finally, the probability of breeding twice within the same season and the probability of bequeathing a territory increased linearly (on a logit scale) from 3 years of age onwards. As resource availability, condition or social status did not increase after 3 years of age in this red squirrel population, we conclude that the proportion of resources allocated to reproduction increased from 3 years of age onwards. As trade-offs between different components of parental energy allocation to reproduction may occur, an increase in one component may be compensated for by a decrease in another one. Therefore, before concluding that total reproductive effort increases, one should have an accurate measure of the whole reproductive effort throughout the reproductive season. In our population, we quantified reproductive effort of females based on seven measures, and the only one that decreased at the end of life was litter size at weaning. As discussed previously, litter size cannot be used to measure reproductive effort because it mostly reflects physiological abilities of individuals and not energy allocated to litter production. Consequently, the increase in propensity to breed twice within the same season and to bequeath territory both give support to Williams's (1966) reproductive value hypothesis: old females increase their allocation to reproduction as an adaptive response to a decrease in reproductive values. To our knowledge, this represents one of the first pieces of empirical evidence supporting Williams' hypothesis in mammals.

Williams' hypothesis has led to the concept of terminal investment (Clutton-Brock 1984). Reproductive investment is an evolutionary concept that corresponds to the energy allocation from the parents that increase the offspring's chance of surviving at the fitness cost of the parent (*sensu* Trivers 1972; Evans 1990). It is generally assumed that an increase with age in reproductive effort reflected an increase in investment, supporting thereby the terminal investment hypothesis (e.g. Mysterud *et al*. 2005; Velando, Drummond & Torres 2006). It seems likely that a given proportion of energy allocated to reproduction will be more costly for senescent females than for prime-ages ones, so that an increase in reproductive effort with age is likely to correspond to an increase in investment when senescence occurs. However, to our knowledge, very few studies have clearly demonstrated that an age-related increase in reproductive effort corresponds to an increase in reproductive investment (but see Pärt, Gustafsson & Moreno 1992 for an example on the collared flycatcher) and empirical evidence supporting the terminal investment hypothesis remains scarce.

METHODOLOGICAL ISSUES LEADING TO DIFFERENT PATTERNS OF AGE-SPECIFIC VARIATION IN REPRODUCTIVE EFFORT

Our study shows that some measures of reproductive effort indicated an increase in the energy allocated at the end of life, whereas some others did not change with increasing age or decreased at the end of life. Similar heterogeneities have been reported in other studies. For example, in female red deer, both birth mass and the probability of producing one offspring are low among young and old animals, whereas suckling bout duration and offspring early survival exhibited opposite patterns (Clutton-Brock 1984). In female bison, mass loss during reproduction does not vary with age, but the probability of producing a young decreases when time spent nursing increases for old females (Green 1990). Factors shaping variation with age in reproductive traits differ according to the trait considered, which emphasizes the complexity of studying age variation in reproductive effort in free-living populations.

Several methodological issues can be advocated to explain the variability in patterns of age variation reported in components of reproductive effort. All these issues are based on the idea of individual heterogeneity. First, if total energy available varies with age, energetic input into reproduction does not represent the actual proportion of energy allocated and is not relevant to quantify reproductive effort. For example, if resource availability increases with age, an increase in litter mass with age may not reflect an increase in reproductive effort. Second, patterns of age-specific variation in reproductive effort at the population level may hide patterns at the individual level (because of within-cohort selection, Service 2000; Cam *et al*. 2002). For example, when females differ in the quality of their territory, a female with a high-quality territory could easily gain mass, whereas a female in a poor territory could gain very little mass despite an intense foraging activity. If the proportion of high-quality females (i.e. females that own a high-quality territory) among each age class increases with age, the result would be an apparent lower mass loss during reproduction as females get older (i.e. pattern at the population level) that can be wrongly interpreted as a decrease in reproductive effort at the individual level. Third, in a situation where reproductive effort is expected to increase as an optimal response to decreasing reproductive value, this decline in reproductive value may itself hamper actual age variation in effort when it is due to senescence (i.e. a deterioration of functional capacities at the end of life, Rose 1991). Senescence can confound the assessment of reproductive effort with age when effort is measured with variables affected by physiological condition of individuals, as it is probably the case for litter mass. For example, old individuals may devote more energy to their reproduction than prime-age ones (e.g. they may spend more time foraging), but may be less efficient at converting energy into offspring or milk production (as suggested in Ericsson *et al*. 2001). The result may be a lower litter mass despite a higher energy allocation from the parent. Thus, when senescence occurs, some measures of reproductive effort commonly used in mammals (from a literature survey we found that 15 of 30 studies addressing age variation of female reproductive effort in mammals were based, at least in part, on litter mass) may not reflect reliably the relative energy allocation to reproduction.

DIFFERENT BIOLOGICAL SITUATIONS LEADING TO DIFFERENT PATTERNS OF AGE-SPECIFIC VARIATION IN REPRODUCTIVE EFFORT

The various patterns of age variation in reproductive effort may also correspond to different biological situations. For example, in a situation where mortality increases in only one age class, the optimal reproductive effort will increase before that age but will decrease after it (Michod 1979). Moreover, in a situation where reproductive effort increases as reproductive value decreases, the energy allocation to reproduction may increase at only some stages of the breeding period and may be expressed only in some traits (Clutton-Brock 1984; Cameron *et al*. 2000). For example, the bequeathal probability increased with age in our population of North American red squirrels but mass change during rearing did not. One explanation could be that increase in reproductive effort for old females is mainly expressed at offspring emancipation because the response of offspring fitness to an extra allocation is the highest at that time. Indeed, by bequeathing their territory to their juveniles, mothers increase the survival of their offspring and thus their recruitment probability (Berteaux & Boutin 2000), which indicates that the response of offspring survival to territory bequeathal is important.

HOW TO STUDY AGE VARIATION IN REPRODUCTIVE EFFORT?

Our study focused on reproductive effort in female mammals, but the same interpretations could be applied to male mammals and to other taxonomic groups. As discussed just above, different biological contexts can lead to different patterns of age specific variation in reproductive effort. Therefore, to address relevant questions and test adequate hypotheses for a particular population, one should first determine how reproductive value varies with age and thus how mortality and reproduction vary throughout life. Then, one should determine whether the population is rapidly growing or not (Charlesworth & Leòn 1976). If population growth rate is close to 0 and if reproductive value regularly decreases with increasing age, one should therefore predict an increase in reproductive effort with age (Roff 2002). Finally, several measures of reproductive effort spanning the whole time period of the breeding cycle are required to get a full understanding of age variation in reproductive effort.

About the methodological issues of the study of age variation in reproductive effort, one should determine whether individual heterogeneity and within-cohort selection is important in the population under study. The use of longitudinal data and mixed models may allow to adjust for such individual heterogeneity (Cam *et al*. 2002; Steele & Hogg 2003; van de Pol & Verhulst 2006). Then, one should use measures of effort not strongly affected by senescence, or complement such measures with behavioural observations. When senescence occurs, the study of individual time budget (especially time spent foraging) might provide a more accurate picture of female reproductive effort than the use of litter mass alone. It has been suggested that 'measurement of reproductive effort should be most preferably based on direct rather than indirect measures' (Roff 2002, p. 235). Our study indicates that if these 'direct measures' are strongly affected by the ageing process and/or by individual heterogeneity, indirect measures, such as behavioural traits (e.g. bequeathal behaviour in our red squirrel population) should be considered in order to assess age-specific variation in reproductive effort.

One key issue in the study of age-specific variation in reproductive effort involves the distinction between patterns due to methodological issues and actual patterns that are of biological importance. Evolutionary hypotheses have often been mixed with methodological issues to explain patterns of reproductive effort. For example, Bowen *et al*. (2006) recently tested whether the 'within-cohort selection hypothesis' (Service 2000) or the 'restraint' hypothesis (Curio 1983) best explained variation in reproductive effort of seals. However, the within-cohort selection refers to the process of individual heterogeneity that should be accounted for to get an unbiased measure of age-specific reproductive effort, whereas the restraint hypothesis refers to an evolutionary hypothesis of patterns of reproductive effort. Likewise, Broussard *et al*. (2005) tried to separate a decrease in effort because of senescence from an increase in effort because of decreasing reproductive values. However, decreasing reproductive values are a direct consequence of senescence in long-lived vertebrates. Such confusions render difficult the assessment of age variation in reproductive effort and we hope our study will put the emphasis on what needs to be done to study reliably age-specific variation in reproductive effort in wild animal populations.

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