Reproductive aging and mating: The ticking of the biological clock in female cockroaches

Patricia J. Moore* and Allen J. Moore

School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom

Females are expected to have different mating preferences because of the variation in costs and benefits of mate choice both between females and within individual females over a lifetime. Workers have begun to look for, and find, the expected variation among females in expressed mating preferences. However, variation within females caused by changes in intrinsic influences has not been examined in detail. Here we show that reproductive aging caused by delayed mating resulted in reduced choosiness by female *Nauphoeta cinerea*, a cockroach that has reproductive cycles and gives live birth. Male willingness to mate was unaffected by variation in female age. Females who were beyond the optimal mating age, 6 days postadult molt, required considerably less courtship than their younger counterparts. Females who were older when they mated had fewer offspring per clutch and fewer clutches than females who mated young. Thus, reduced choosiness was correlated with a permanent reduction in fertility. There was no difference in overall senescence among females, and thus the reduction in clutch size did not result in the expected increased lifespan. We suggest that reproductive aging in *N. cinerea*, similar to aging in general, occurs because the maintenance of oocytes is costly, and selection is relaxed after the optimal mating period. Our results further suggest that selection for continued choosiness is also relaxed and supports direct selection on female choosiness and a cost to choosiness.

aging | female mate choice | fitness | mate quality | sexual selection

Sexual selection theory predicts that mate choice, usually by females, evolves because of variation in benefits provided by mating with particular mates. Much of the recent research on sexual selection therefore has been directed at detecting the benefit provided by males to females (reviewed in refs. 1 and 2). Considerably less research effort has addressed variation in female preferences (3–7). During female mate choice, however, females must tradeoff the benefits gained by being choosy against the costs of stronger preferences (2). This cost/benefit tradeoff should result in variation in the expression of female mate preferences both between females and by an individual female over her lifetime.

Variation in female mate preferences can result from extrinsic or intrinsic factors. Examples of external conditions that may cause females to be less choosy when making mating decisions include environmental conditions that increase the energy output required to search for and compare different males (8) or high predation risk (9–11). Seasonal constraints on reproduction can also influence female choosiness (12).

Intrinsic factors should also influence the expression of female mate choice (13, 14). Parker's ESS model of mate choice (13) suggests that the level of choosiness is a function of female reproductive quality. Females with a reproductive quality above a certain threshold should only accept mates of similarly high quality. However, females with a reproductive quality below this threshold should be less choosy, accepting lower quality males as mates. Parker's model considers reproductive quality as an intrinsic value of the female: what happens if female reproductive quality changes over time? Is there phenotypic plasticity in female choosiness based on

reproductive state? Few empirical studies have addressed these questions. Lea *et al.* (15) present evidence that the consistency of mate preference in midwife toads, presumably reflecting a high motivation to mate, is greatest in ovulating females. Kodric-Brown and Nicoletto (16) find that older female guppies are less choosy than when they are younger even if still virgin. Likewise, Gray (17) demonstrated that older female house crickets show no significant preference for the calls of attractive males compared with young females.

An essential factor in considering the effect of reproductive state on the expression of female mate choice is to show that in fact there is variation in the costs associated with mate choice that are correlated with changes in reproductive state. None of these previous studies (15–17) addressed whether there is an associated cost to maintaining mate choice. In fact, Lea *et al.* explicitly state that there is no additional cost to maintaining choosiness for ovulating females of the Mallorcan midwife toads, because they can hold eggs for about a week in their oviducts and thus can afford to be discriminating.

The study we report here addresses the question of how female mating preferences vary as the cost of choice changes. We show that in the cockroach *Nauphoeta cinerea* the choosiness of females, defined as the courtship effort females require from males before mating, changed as a function of female age because of age-related changes in the reproductive system. Older females were less choosy than younger females. We also showed that the decrease in choosiness was correlated with a reduction in the reproductive potential of older females, providing the first evidence of varying costs of mating preferences reflecting intrinsic changes in a female.

N. cinerea Life History

N. cinerea is ovoviviparous; although no nutrients are passed from the mother to the developing young, females carry the developing embryos in a brood pouch until the offspring emerge as first instar nymphs (18). Females may also provide maternal care in the form of protection to the offspring during their first instar (ref. 19; unpublished data). Thus, there is a relatively large maternal investment in *N. cinerea*. Once a female has mated, she is unreceptive to males until the birth of her first clutch (20, 21). Even then, females may not remate. Maternal care further limits the number of offspring a female can produce (21, 22). In contrast, males provide no paternal care and make only genetic contributions to their offspring (22, 23). Thus, compared with female *N. cinerea*, males have a relatively unlimited fertilization capacity (20–23). This asymmetry in parental investment is expected in species in which sexual selection, and particularly mate choice, operate (24).

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Sexual selection in *N. cinerea* occurs as a result of both male–male competition and female mate choice (reviewed in ref. 25). Preferences by females are reflected in their willingness or unwillingness to mate (25, 26), and mate choice can be independent of male-male competition (27, 28). Further, female behavior toward single males is no different from their behavior when multiple males are present, indicating that *N. cinerea* females use a threshold mating preference (22). Female choosiness during courtship has been shown to reflect mating preferences and result in mate choice in *N. cinerea* [refs. 22, 28, and 29; our definitions follow Jennions and Petrie (2) where "choice" refers to the outcome of the propensity to mate, and "preference" and "choosiness" refer to the amount of time spent assessing mates]. Females require significantly less courtship effort from preferred males regardless of the number of males present (22, 28, 29).

Materials and Methods

Animal Husbandry. Late instar nymphs were isolated from mass colonies, separated according to sex into $17 \times 12 \times 6.5$-cm plastic containers, and provided with *ad libitum* rat chow and water. All animals were kept in incubators at 27°C on a 16/8 light/dark cycle. Adult females and males were isolated on the day of adult emergence, and individuals were placed into $11 \times 11 \times 3$-cm clear plastic boxes and provided with food and water.

Mating Trials. All males used in the mating trials were 10 days postadult emergence. Individual males were only used once. Females at 3, 6, 9, 12, 15, or 18 days postadult emergence were placed with a randomly selected male in a $17 \times 12 \times 6.5$-cm clear plastic box. As noted above, variation in choosiness in *N. cinerea* is expressed as the variation in timing of female responses to male courtship, with females mating more quickly with preferred mates.

Courtship behavior was observed and timed as described in detail in ref. 29. Briefly, courtship in *N. cinerea* occurs after the female is attracted to the male from a distance by a male-produced pheromone. The male responds to the presence of a female by raising his wings and exposing his abdomen. If the female is receptive, she responds to the male's wing-raise by climbing on the male's abdomen. The male extends his abdomen attempting to grasp the female's genital opening with his pygidium, an internal hook-like structure. Females can avoid mating by simply backing off of the male's abdomen, thus making her genitals unavailable. Once the male has grasped the female, he rotates 180° such that mating is in the end-to-end position typical of cockroaches. Copulations terminate when the male releases the female.

If a female refused to mate with a male within 5 min of the start of the mating trial, a time sufficient to indicate long-term unwillingness to mate (26), she was placed back into her individual container and isolated for 24 h. Such females invariably were unresponsive to male courtship altogether rather than responding to courtship but refusing copulations. Females that had failed to respond to males were placed into another mating trial the next day with a new male (10 days old). This continued until the female mated. All females in the study eventually mated.

Reproductive Output. After mating, females were housed in $11 \times 11 \times 3$-cm clear plastic boxes and provided with ad libitum food and water. Females were checked daily for death and offspring. For each clutch, the date of parturition and the number of offspring were recorded. Offspring were returned to the mass colony and were not housed with the female. After we removed the offspring we returned the females to their individual containers, in which they were allowed to give birth to subsequent clutches until they died.

Statistical Analyses. Standard parametric (ANOVA) and non-parametric (goodness-of-fit) tests of significance were used, because our data were either normally distributed or met distribution requirements after transformation. Within ANOVA, we tested *a priori* predictions that change is associated with age by using contrast analysis (30). We tested four specific contrasts based on the pattern of reproductive maturation and receptivity in this species, in which an increasing number of females are receptive up to 6 days postreproductive molt, but all females are receptive after 9 days as adults (21). Thus, in addition to the overall ANOVA, we tested for a linear change with age, a deviation from linearity (quadratic change with age), significant differences between young and old ages (3, 6, and 9 differ from 12, 15, and 18), and significant differences among three groups, young, middle and old ages (3 and 6 differ from 9 and 12, which differ from 15 and 18). All statistical tests were performed by using SYSTAT 9.0.

Results

Effects of Age on Mating Behavior. Only 6 of 20 females presented with males at 3 days postadult emergence mated on the first day. Of the 14 females that did not mate on day 3, 10 mated on day 4, one on day 5, and the remaining two females mated on the sixth day postadult emergence. Of the females presented to males for the first time 6 days postemergence, 17 of 20 mated on the first day. The remaining three mated at the age of 7 days of age. All other females (9, 12, 15, and 18) mated on the first day a male was offered as a mate. These data correlate well with previous work showing that receptivity is fully developed by 7 days postadult emergence (21).

In those interactions that resulted in matings and fertilizations, there was a significant effect of age on the total length of courtship (Fig. 1*A*; ANOVA, $F_{5, 114} = 8.713$, $P < 0.001$). There was both a significant linear decrease in the amount of courtship required by increasingly older females ($F_{1, 114} = 25.707$, $P < 0.001$) and significant deviation from linearity ($F_{1, 114} = 13.040$, $P = 0.0005$). There was a significant difference between young and old groups of females ($F_{1, 114} = 13.336$, $P = 0.0005$) and between young, middle, and old age groups ($F_{1, 114} = 21.118$, $P = 0.0004$).

The decreased courtship required by increasingly older females reflected a significant difference in the time females took responding to male courtship displays (Fig. 1*B*; ANOVA, $F_{5, 114} = 11.380$, $P < 0.001$). There was a significant linear trend toward shorter responses by older females ($F_{1, 114} = 43.316$, $P < 0.001$) and a significant deviation from linearity ($F_{1, 114} = 8.568$, $P = 0.004$). Females grouped into old and young categories were significantly different ($F_{1, 114} = 28.467$, $P < 0.001$), as were females grouped into young, middle, and old categories ($F_{1, 114} = 35.388$, $P < 0.001$). In contrast, once the female responded to the male, there was no significant effect of female age on the time between a female response and the start of copulation (ANOVA, $F_{5, 114} = 1.016$, $P = 0.412$).

Males responded to all females regardless of the age of the female at the time of mating (Fig. 2). There was no significant difference in the time it took a male to initiate courtship with females of different ages (ANOVA, $F_{5, 112} = 1.581$, $P = 0.171$; power = 0.524). In addition, the female's age at mating did not have a significant effect on the length of copulation (ANOVA, $F_{5, 111} = 1.630$, $P = 0.158$; power = 0.561).

Effect of Age on Reproductive Output of Females. Females that mated at 15 or 18 days postadult emergence took much longer to produce a first clutch than younger females (Fig. 3*A*; ANOVA, $F_{5, 105} = 7.711$, $P < 0.001$). There is a significant linear increase ($F_{1, 105} = 19.395$, $P < 0.001$) as well as a significant deviation from linearity ($F_{1, 105} = 15.209$, $P < 0.001$). There was a significant difference between females grouped into young and
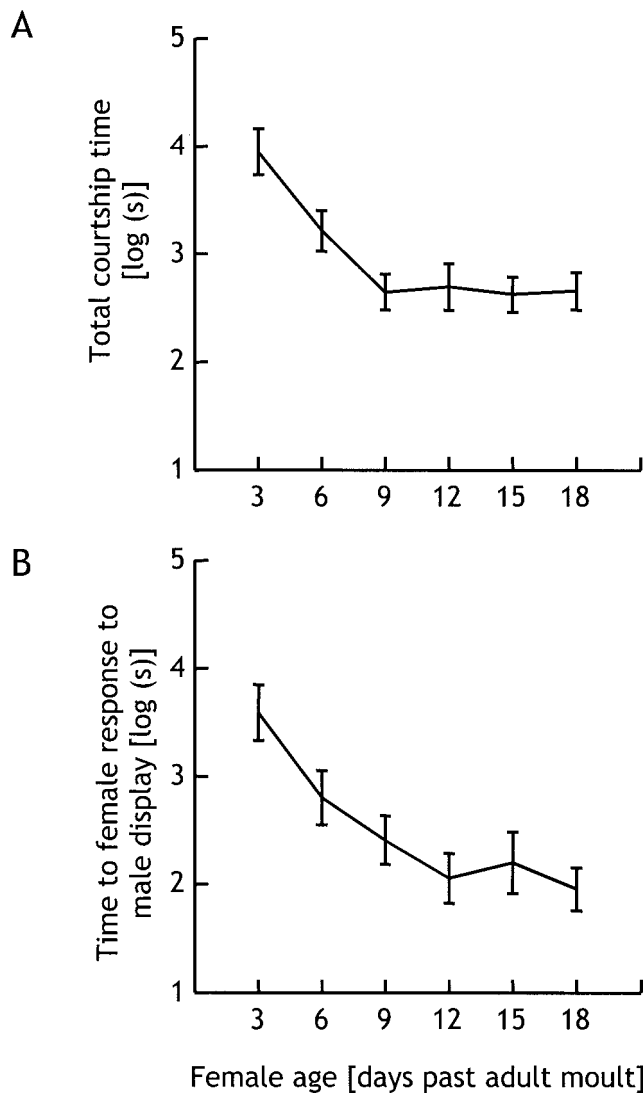
Fig. 1. Female response (log-transformed, s) to male courtship at different ages. (*A*) Total courtship time was longer in younger females than in older females. This reflects the longer time it took for younger females to respond to the males' courtship display (*B*). Sample sizes for females mated at 3, 6, 9, 12, 15, and 18 days postadult molt were 20, 19, 20, 20, 21, and 20, respectively.
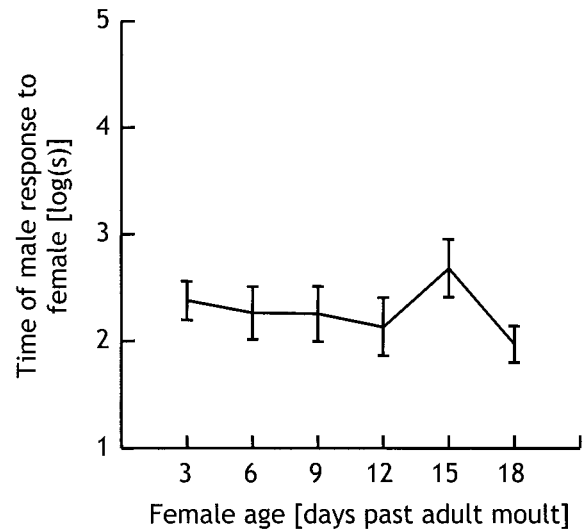

Fig. 2. Males respond equally to females at all ages (log-transformed, s). Sample sizes for females mated at 3, 6, 9, 12, 15, and 18 days postadult molt were 20, 17, 20, 20, 21, and 20, respectively.

old categories ($F_{1, 105} = 8.009$, $P < 0.006$) and females grouped into young, medium, and old categories ($F_{1, 105} = 17.226$, $P < 0.001$). This effect was most obvious in the first clutch for females mated at 18 days of age. In females mated at 3, 6, 9, and 12 days postadult emergence, only 1 of 75 took greater than 60 days to give birth to their first clutch. Of the females mated at 15 days postadult emergence, 3 of 18 took greater than 60 days to give birth to their first clutch. In females mated at 18 days postadult emergence, nearly two-thirds, 11 of 18, took greater than 60 days to give birth to the first clutch. The length of time between the first and second clutch was unaffected by the age of the female when mated (ANOVA, $F_{5, 84} = 0.856$, $P = 0.514$; power = 0.503), although there was a trend for a delay in females mated at 18 days of age (Fig. 3*B*).

The age of the female when mated had a significant effect on the number of offspring she produced in the first clutch (Fig. 4*A*; ANOVA, $F_{5, 105} = 6.719$, $P < 0.001$). There was a significant linear decrease ($F_{1, 105} = 31.961$, $P < 0.001$) and no significant

deviation from linearity ($F_{1, 105} = 1.064$, $P = 0.305$). Groups of young and old females were significantly different ($F_{1, 105} = 29.4182$, $P < 0.001$), as were females categorized into young, medium, and old groups ($F_{1, 105} = 28.273$, $P < 0.001$). The same pattern was seen in the second clutch, although with less extreme differences between the age groups (Fig. 4*B*; ANOVA, $F_{5, 90} = 2.399$, $P = 0.043$). There was a significant linear decrease ($F_{1, 90} = 8.715$, $P < 0.004$) and no significant deviation from linearity ($F_{1, 90} = 0.012$, $P > 0.912$). Young and old female groups were significantly different ($F_{1, 90} = 11.4064$, $P < 0.002$), as were females categorized into young, medium, or old groups ($F_{1, 90} = 8.363$, $P < 0.005$).

The age at which females first mate had a highly significant effect on lifetime fertility (Fig. 4*C*; ANOVA, $F_{5, 104} = 7.295$, $P < 0.001$). There was a significant linear decrease in fertility with increasing age ($F_{1, 104} = 31.848$, $P < 0.001$) and no significant deviation from linearity ($F_{1, 104} = 2.928$, $P > 0.09$). Females categorized into young and old groups were significantly different ($F_{1, 104} = 21.599$, $P < 0.001$), as were females categorized into young, medium, or old groups ($F_{1, 104} = 30.850$, $P < 0.001$). The effects on total fertility reflected both a reduction in the number of offspring per clutch and fewer clutches, because there was a significant effect of the age mated on average clutch size (ANOVA, $F_{5, 104} = 6.874$, $P < 0.001$) and a significant association between the age mated and total number of clutches produced ($\chi^2 = 20.608$, df = 10, $P = 0.024$).

Although there was a reduction in overall fertility when females mated at an older age, there was no effect on female lifespan associated with different ages of mating (ANCOVA, $F_{5, 95} = 0.984$, $P = 0.432$; power = 0.466). The average clutch size was a significant covariate of longevity in this analysis ($F_{1, 95} = 7.085$, $P = 0.009$). For all groups except females mated at age 18, smaller clutches resulted in a longer lifespan (Fig. 5). Thus, in very old females, reduced fertility associated with aging did not result in a longer lifespan.

Discussion

We have shown that female *N. cinerea* became less choosy as they age; that is, older females mated more quickly than younger females. Delay in mating is one of the mechanisms that females of this species use to express preferences among males, resulting in mate choice (22, 28, 29). Thus, decreased choosiness will result
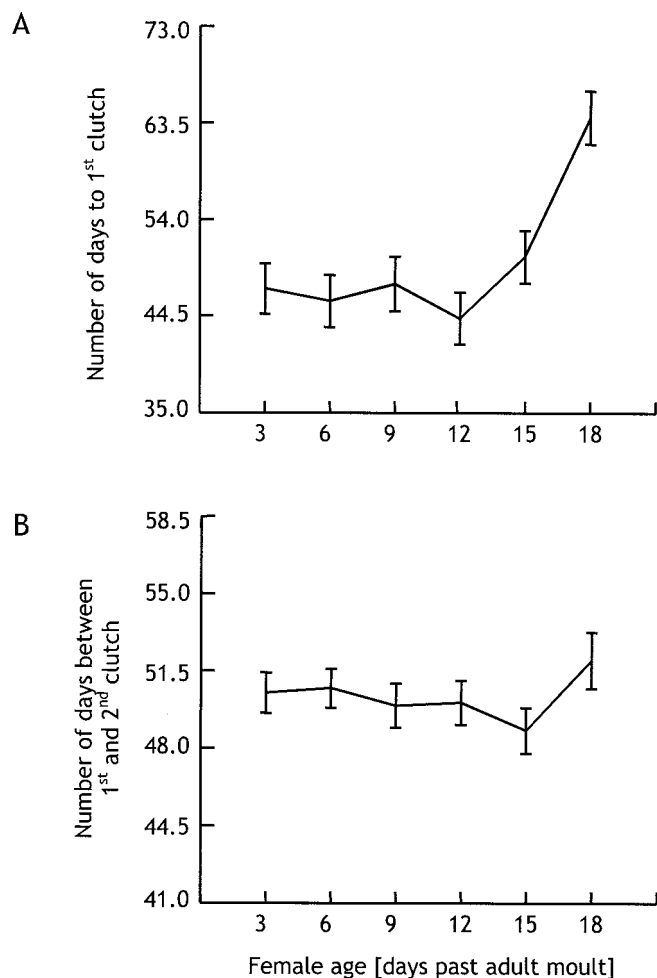
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Fig. 3. Offspring of females mated at an older age take longer to develop than the offspring of females mated at a younger age. (*A*) The time to parturition of the first clutch is delayed in females mated 15–18 days after adult emergence. (*B*) There is not a significant delay between the first and second clutch, although there is a trend for a longer period between clutches for females mated at 18 days postadult emergence. Sample sizes for females mated at 3, 6, 9, 12, 15, and 18 days postadult molt were, respectively, 20, 19, 17, 19, 18, and 18 for timing of the first clutch and 18, 19, 15, 15, 14, and 9 for timing of the second clutch.

Fig. 4. The age when first mated has a significant effect on the number of offspring. (*A*) The number of offspring in the first clutch is significantly reduced in females mated 12–18 days postadult emergence. (*B*) There is a trend toward lower numbers of offspring in the second clutch of females mated 12–18 days postadult emergence, although the effect does not reach conventional levels of significance. (*C*) The total number of offspring in these females is significantly reduced. Sample sizes for females mated at 3, 6, 9, 12, 15, and 18 days postadult molt were, respectively, 20, 19, 17, 19, 18, and 18 for the number of offspring in the first clutch and total number of offspring and 18, 19, 16, 15, 16, and 12 for the number of offspring in the second clutch.

in reduced mate choice. We also showed that decreased choosiness was correlated with a permanent decline in the reproductive quality of females. Therefore, as females age past an optimal breeding period, the cost of mating preferences increased rapidly if preferences delayed mating. For changes in both choosiness and reproductive fitness, the changes were primarily linear with increasing age. In general, beyond the age at which all females are receptive and therefore likely to have mated, fitness and choosiness began to decline sharply. These effects were particularly pronounced in females 18 days postadult molt, the oldest females we tested.

In contrast to the female behavior, we found that male courtship and mating behavior showed no obvious change as a function of female age. Male choosiness will only be favored if the cost of searching for a new mate (including the potential of not finding another female) is less than the cost of time spent in copulation and of replenishing sperm (13). Under our experimental conditions, perhaps males were unable to assess female age and reproductive quality or that the cost of passing up even a poor mating opportunity was greater than the investment in time and sperm production.
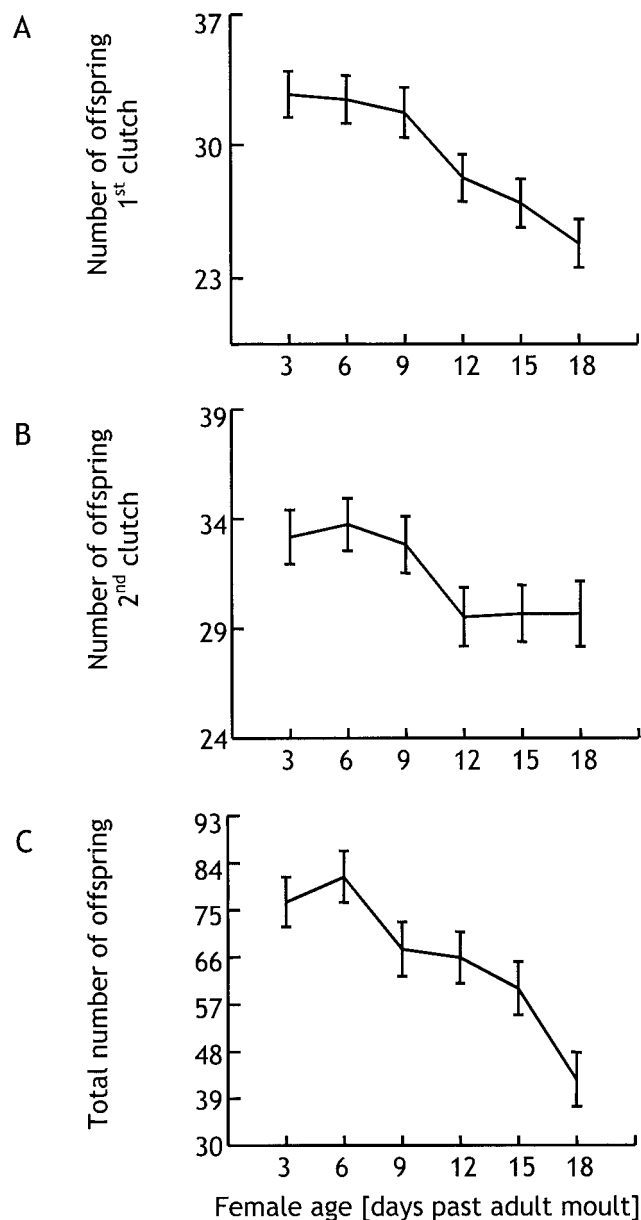
Reproductive Senescence and Mate Choice. The reproductive aging that we saw has some correspondence with overall senescence. Female *N. cinerea* in our experiments lived for 150–200 days regardless of age of mating. However, in this experiment we found that average clutch size was a slightly positive covariate with lifespan considering the different treatments. This was in contrast to the negative relationship between average clutch size and longevity we see in multiple experiments and under multiple social conditions (ref. 31; unpublished results). The effect in
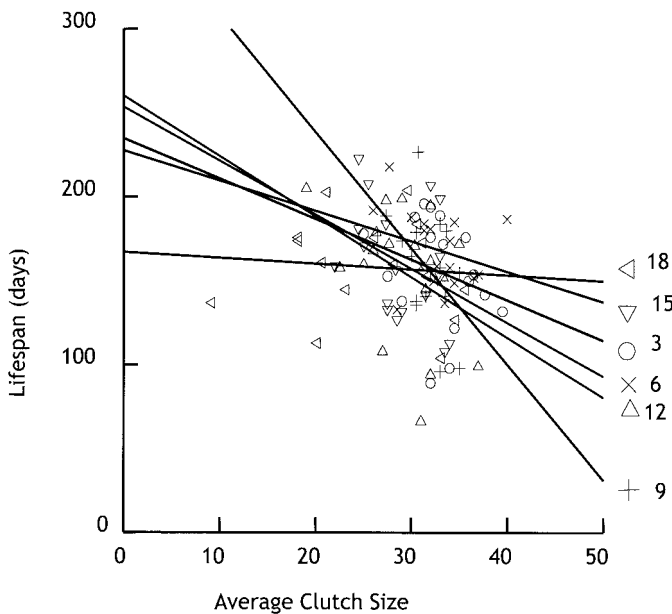
Fig. 5. The tradeoff between average size of clutch produced and total lifespan for females mated at 3 ($n = 17$), 6 ($n = 18$), 9 ($n = 15$), 12 ($n = 18$), 15 ($n = 18$), and 18 ($n = 16$) days postadult molt is depicted.

the present study was driven by the lack of increased lifespan in females mated at 18 days of age despite their decreased fertility. Thus, females mating at an older age are suffering reduced fecundity without gaining the expected extended lifespan.

Reproductive senescence in *N. cinerea* is likely to be correlated with changes in the reproductive cycle of females as they enter sexual maturity. Newly emerged females are not receptive to mating. During the first 4 days, neurosecretory materials accumulate and activate a receptivity center in the brain, leading to the development of mating behavior (32). During this time the corpus allatum is inhibited by nervous signals from the brain, leading to low levels of oocyte maturation and yolk deposition (33). The result of the coordinated activities of the receptivity center and the corpus allatum is that females typically mate when oocytes are small, about 1-mm long, and some yolk has been deposited. The insertion of the spermatophore after mating acts to reduce receptivity and increase activity of the corpus allatum, stimulating the further growth and development of the oocytes (34). Thus, about 7 days after mating, mature oocytes pass through the bursa past the spermatheca and are fertilized, packaged into an ootheca, and retracted into the female's brood pouch for the period of gestation.

By preventing females from mating at the "optimal" point in the initial reproductive cycle, presumably around 6 days postadult emergence, we have somehow interrupted the carefully coordinated interplay between the receptivity center in the brain and the hormonally controlled development of the oocytes. It might be argued that the oocytes in this first cycle were simply damaged in some way by the mistiming of oocyte maturation, and thus the number of offspring in the first clutch was reduced because of a reduction in the number of viable oocytes. We argue against this for two reasons. First, during gestation, maturation of the oocytes that will give rise to the next clutch is inhibited by the presence of the ootheca in the brood pouch in a manner similar to that in which oocyte maturation is inhibited before mating (33) with no effects on viability of the second clutch. Second, the effects of late mating, although having the greatest impact on the first clutch, continued to be seen in subsequent clutches, each of which showed a pattern of reduced numbers of offspring (Fig. 4) that led to overall reduced fertility over the lifetime of the female.

Clearly there can be a cost to remaining choosy given the linear and permanent reduction in fecundity with individuals mated at an increasingly older age. The question is, then, why does this decline in fecundity, and thus reproductive senescence, occur? An optimality model by Begon and Parker (35) considers how reproduction should change as females age and suggests that reproductive output should decline with time because of resource limitation. This does not explain our results, because the period of our study, less than one reproductive cycle, was too short for resource limitation to apply. Rather, we suggest there must be a cost to retaining viable oocytes, and this therefore suggests a mechanism of reproductive senescence. If all females are normally mated by 6 days of age, selection for oocyte maintenance may be relaxed beyond this point. We also suggest that there has been no selection for mating preferences beyond the optimal age, because females are normally unlikely to remain unmated. The decay in female choosiness with age is therefore not necessarily adaptive. Rather, the decay reflects a lack of selection to maintain costly choice. Our ideas on reduced selection beyond an optimal age is similar to hypotheses regarding the evolution of senescence, and borrows from Fisher's argument (36) that aging begins when reproductive value begins to decline because of relaxed selection. Considerable research on aging shows that the efficiency of selection can diminish with age (37).

The relationship between the correlated changes in choosiness, costs, and decline in reproductive value may be clearer for reproductive senescence than for overall aging. Accumulation of late-acting mutations seems unlikely, given that reproductive aging is occurring over a time span of days in an organism that will live for months. More likely a tradeoff between maintaining current and future oocytes limits reproduction by older females. In addition, pleiotropy between choosiness and reproductive senescence remains a possibility. Partial support for this last idea is seen in artificial selection for increased lifespan in *Drosophila*, in which slower mating (38) and increased choosiness (39) are seen as correlated responses to increased lifespan.

Reduced selection with reduced reproductive value also argues for a cost to choice and direct selection on choosiness. As selection for maintaining viable zygotes is relaxed so too will be selection for choosiness. Few studies have been able to demonstrate direct selection on mate choice without identifying factors limiting the expression of choice (e.g., predation and energetic costs). Our study shows that internal factors such as female condition or reproductive state need to be considered in calculations of costs and benefits as well.

Researchers are beginning to look for and identify variation among females in patterns of mate choice (3–7). As we show here, variation may occur within a female as well as between females if female condition changes. Such changes may be common, because a similar pattern of reduced reproductive capacity with age is seen in organisms taxonomically distinct from cockroaches such as humans (40). We now need studies that help identify the costs of maintaining choosiness and reproductive value. *N. cinerea* may provide a simple system for investigating the costs and mechanisms involved in reproductive aging in a species with reproductive cycles. This also offers a convenient insect species for studies of aging that can complement studies of insects with continuous reproduction (e.g., see ref. 41).

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