Satu Paukku

Cost of Reproduction in a Seed Beetle

A Quantitative Genetic Perspective

ABSTRACT

Life-history theory predicts a trade-off between traits that have an influence on fitness; an example of such trade-off is between reproduction and longevity. In order for the trade-off between reproduction and other life-history traits to evolve, there must be variation in how individuals tolerate the cost of reproduction. In this thesis, I examined whether female reproductive decisions affect offspring fitness in *Callosobruchus maculatus* seed beetles. It seemed that females tend to lay their eggs more or less randomly. However, there was a difference in the distribution of eggs between the bean types, suggesting that females make some oviposition decisions depending on the bean type. More importantly, I examined the cost of reproduction in male *Callosobruchus maculatus* seed beetles. I found a cost of reproduction that can be seen as reduced longevity. Furthermore, I studied whether the tolerance of cost of reproduction varies between families. I found that there was genetic variation in the tolerance of reproductive costs in *Callosobruchus maculatus* seed beetle males. A significant interaction between reproductive effort treatment and the genotype on the longevity of males indicated that the variation in tolerating the cost of reproduction has a genetic basis. Moreover, I examined, whether the genetic variation in ejaculate size could be the explaining factor for genetic variation in the cost of reproduction. I show that there is genetic variation in the ejaculate size, and also covariance between the ejaculate size and longevity. Besides that, those genotypes that live longer without reproduction, have larger ejaculate sizes and thus higher cost of reproduction. This means that genotypically differential investment in ejaculates is likely to explain the observation of the genetic variance in tolerating the cost of reproduction in *C. maculatus* males. All these findings suggest that male reproductive effort is far more complex than has traditionally been thought.

Key words: Bruchidae; cost of mating; fitness; life-history trait; quantitative genetics; seed beetle; trade-off.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original studies which will be referred to in the text by their roman numerals (I-IV). I am the main author in all papers, and I carried out a large part of the planning and data collection in each paper.

I   Paukku, S. & Kotiaho, J. S. The effect of female reproductive decisions on progeny fitness. Manuscript (submitted).

II   Paukku, S. & Kotiaho, J. S. 2005. Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity. J. Insect Physiol. 51: 1220-1226.

III Paukku, S., Kotiaho, J. S. & Tomkins, J. T. Genetic variation in tolerating the cost of reproduction in male *Callosobruchus maculatus*. Manuscript.

IV   Paukku, S., Kotiaho, J. S. & Tomkins, J. T. Ejaculate size explains the genetic variation in the cost of reproduction for male seed beetles. Manuscript.

1 INTRODUCTION

1.1 Difficulties in maintenance of genetic variation

Evolution cannot progress without additive genetic variation (Roff 1997). This is why genetic variation of life-history traits is an important subject to study. Ultimately, genetic variation is generated by mutations (e.g. Roff 1992, Hartl & Jones 2002) but there are also other factors affecting the amount of genetic variation (Roff 1992, Roff 1997). Life-history theory proposes alternative mechanisms for the maintenance of genetic variation in life-history traits (Roff 1992, Roff 1997). Here I present three main mechanisms.

The first mechanism is mutation-selection (MS) balance. It means that there exists a balance between those components that create genetic variation and components which remove variation (Roff 1997). In other words, in the MS balance situation, mutations create as much new genetic variation as selection erodes (Roff 1992, Houle 1998).

In the second mechanism, fluctuating selection, the optimal phenotype can vary in space or in time (Hamilton & Zuk 1982, Schluter et al. 1991). In this case, alleles have environmentally dependent effects on fitness (Tomkins et al. 2004). Under this mechanism, there may be variation in selection or in phenotype.

The third mechanism is negative (antagonistic) pleiotropy. It means that a gene has a positive effect on one fitness component but a negative effect to another component (Roff 1992). This way there forms a negative correlation between these components. In negative pleiotropy, one gene may also be advantageous for young individual but deleterious for old one (Roff 1997). Furthermore, negative pleiotropy is the genetic basis of trade-offs, and thus it is important for life-history theory (Roff 1992).

1.2 Reproductive trade-offs

One of the most important factors to drive evolution of life-histories is a trade-off situation between traits that have an influence on fitness (Williams 1966, Reznick 1985, Roff 1992, Tatar et al. 1993, Messina & Slade 1999, Koivula et al. 2003, study II). The trade-off situation between reproduction and another life-history trait is formed, when investment to reproduction reduces longevity, growth, future fecundity or for example immune function (Williams 1966, Partridge & Harvey 1985, Fowler & Partridge 1989, Roff 1992, Siva-Jothy et al. 1998, McKean & Nunney 2001, Rolff & Siva-Jothy 2002, Kotiaho & Simmons 2003, Messina & Fry 2003). Reproduction can have negative effects to another life-history trait. This kind of trade-off situation is called a cost of reproduction. A reproductive trade-off arises more detectable, when there is only a limited amount of resources to use (Fowler & Partridge 1989, Roff 1992). In other words, when energy is allocated to one life-history trait e.g. reproduction, it precludes possibility to invest these resources to another life-history trait e.g. longevity.

Reproduction incurs costs for both females and males, and for females, costs of reproduction are often documented (e.g. Fowler and Partridge 1989, Tatar et al. 1993, Westendorp & Kirkwood 1998, Messina & Slade 1999). Overall, the cost of reproduction for females can be composed of several factors including tolerating harassment and courtship by males, copulation, harmful effects of male seminal products, producing eggs, pregnancy, giving birth, parental care or, as is most likely, some combination of these factors (e.g. Roff 1992, Kotiaho & Simmons 2003). For example, for *Callosobruchus maculatus* seed beetle females, reproduction is costly in terms of reduced longevity (e.g. Fox 1993, Messina & Slade 1999). The cost is composed of copulation and egg production but it has also been suggested that mating causes damages that may contribute to the cost of reproduction (Crudgington & Siva-Jothy 2000).

The cost of reproduction for males on the other hand, has been studied much less than that in females. Historically, it was assumed that sire invests in offspring only by copulating and donating cheap sperm with its genes (see Simmons 2001). However, there is evidence from *Drosophila melanogaster* fruit flies and *Hygrolycosa rubrofasciata* wolf spiders that, for example, courtship alone is capable of reducing the longevity of males (Cordts & Partridge 1996, Mappes et al. 1996, Kotiaho 2000, see also Kotiaho 2001). The cost of reproduction for the male may be caused among other things by sperm and ejaculate production, contests between males, courtship, copulation, injuries caused by females and parental care (e.g. Kotiaho 2001, Kotiaho & Simmons 2003, Kortet & Hedrick 2005). Despite the limited empirical evidence, it is likely that there are nearly always also some costs arising from the reproductive effort for males.

Besides costs, there are also benefits of reproduction. For example, in several insect species females may get benefit of received nutrients as a nuptial

gift in form of prey item, or nutrition containing ejaculates or spermatophores, or as sexual cannibalism when copulating male is a kind of nuptial gift because female eats him or parts of him before or during the copulation (e.g. Vahed 1998, Wedell & Karlsson 2003), even though the effect of nuptial gifts on female fecundity is not always so straightforward (Maxwell 2000). Furthermore, a nuptial gift may be also beneficial for a male, if it prolongs copula duration and thus maximises ejaculate transfer (Vahed 1998). Prey item as a nuptial gift may also save male from sexual cannibalism (Bilde et al. 2006). Finally, it is worthwhile to remember that reproduction is beneficial for all animals as reproduction is needed if wanting to transfer one's genes to next generation.

1.3   Genetic variation from quantitative genetic perspective

In order for life-history traits to evolve, there must be genetic variation in how reproduction is tolerated (Roff 1992). According to life-history theory, there are two sources of genetic variation that influence total fitness: variation in resource acquirement (i.e. condition) and variation in the allocation of resources among fitness components (Rowe & Houle 1996, Hunt et al. 2004, Tomkins et al. 2004). The tolerance of the cost of reproduction is likely to vary between sexes but also between individuals of the same sex. The latter variation is of interest because it may reflect genetic variation in the tolerance of the cost of reproduction, although there may naturally be also environmental causes for this variation. Therefore, an experimental set up with a planned breeding design and a manipulated reproductive effort is needed if wanting to solve whether there is variance in tolerating the cost of reproduction and whether this variation has a genetic basis. Furthermore, studying the genotype by environment interaction and the reaction norms, i.e. the several phenotypes that a certain genotype may express depending on the environment, it may reveal genetic differences in fitness across the genotypes (Falconer 1989, Gomulkiewicz & Kirkpatrick 1992, Lynch & Walsh 1998, Hartl and Jones 2002, Hunt et al. 2004, Tomkins et al. 2004).

Heritability and genetic correlation are two important parameters in quantitative genetic theory. Heritability estimates the resemblance between relatives, e.g. between parents and offspring (e.g. Falconer 1989, Roff 1992). In this thesis, I have used term heritability as meaning the narrow-sense heritability (V_A/V_P). Furthermore, genetic correlation measures the extent to which different traits are genetically coupled (Roff 1992). Genetic correlation can also be calculated within a trait between two environments, when this trait should be considered as two different traits (Lynch & Walsh 1998). In this thesis, I calculated genetic correlations according to Roff (1997).

1.4 Aims of the study

There were four main aims in this thesis all concentrating on the cost of reproduction in *Callosobruchus maculatus* seed beetles. First, I examined the effect of female reproductive decisions on offspring fitness, i.e. whether a female tends to avoid larval competition among her offspring, and furthermore, whether larval competition reduces some life-history traits (I). The hypothesis was that larval competition causes costs for offspring, and that dam would avoid formation of these costs.

However, there is growing evidence that male reproductive effort is more complex than has traditionally been thought. This is why my second aim was to determine, whether there exists the cost of reproduction in terms of reduced longevity also in *C. maculatus* males (II).

The third aim of this study was to determine whether there exists genetic variation in the tolerance of the cost of reproduction in males (III). Tolerance of the cost of reproduction means that some individuals are more sensitive to the cost of reproduction than others. In other words, there is a certain cost of reproductive investment for all reproducing individuals, but some individuals can tolerate this cost better than others, giving them a less forceful effect of this investment. This experiment I performed with methods of quantitative genetics in a half-sib breeding design. As I found that there was genetic variation in tolerating the cost of reproduction, the fourth aim of this thesis was to solve what was the mechanism to cause this. These beetles loose a huge amount of energy and/or water in ejaculates. Thus, I studied genetic variation in the ejaculate size and the effect of ejaculate size on male longevity (IV).

2 MATERIAL AND METHODS

2.1 Study species

Callosobruchus maculatus seed beetles live naturally in tropical and subtropical regions. *C. maculatus* is a common post-harvest pest of legumes (Fox 1993). Dams lay their eggs on several species of *Vigna*-beans, e.g. black-eye beans (*V. unguiculata*, also known as cowpea) and mung beans (*V. radiata*). 4–5 days after egg laying, first instar larvae burrow themselves inside the bean. Larvae develop inside the bean to adults in an average of 25-40 days (Fox 1993, Fox & Tatar 1994, Savalli & Fox 1998). However, development time is strongly dependent on environmental factors, such as temperature. Both sexes can copulate soon after emerging and both sexes can copulate several times during their life time. The population used in these experiments was derived from a laboratory culture that was originally established from animals collected from Brazil. This population has been maintained on black-eye beans (*V. unguiculata*) in laboratory since 1982.

In this species, both sexes are active in copulations. According to my observations, the male searches a partner who he copulates with and male is on the top when copulated. However, a female may prevent male from copulating by kicking him when he tries to approach her. Females also seem to decide the copula duration because they activate a male to detach from her by kicking him (Edvardsson & Tregenza 2005). Copula duration of males in my experiments was around 5-30 minutes (own observation). Females of this species have been shown to suffer genital damages because of male genitalia (Crudgington & Siva-Jothy 2000).

2.2   Environmental conditions during experiments

Animals were cultured in containers of 0.9 litres (95 x 95 x 100 mm) with about 500 dried black-eye beans in each container. Culture containers were pierced to ensure supply of air. Culture and animals in experiment were kept at 20 °C in the laboratory, where light conditions were 15L:9D during experiments of studies I, II and the first experiment of study III, but they were kept in heated laboratory or incubator at 28 °C, and 12L:12D light period in the second and third experiment of study III and in experiments of study IV. Adults of this species do not need nutrition or water to reproduce, but they collect all necessary energy reserves during their larval stage (e.g. Fox & Tatar 1994), even though adults received extra nutrition may get benefits, e.g. in prolonged lifespan (Tatar & Carey 1995). Because of this, at the moment of their emergence these beetles have a fixed amount of energy to use during their adult life. Thus, this species is ideal for manipulative studies of trade-offs.

2.3   Procedures for larval competition experiments

I defined the female oviposition preference in relation to host species, mung and black-eye beans, by letting a female to lay eggs inside a container which contained an equal amount of both bean species (I). This set-up was repeated 100 times.

   I studied larval competition with the same two bean host species, mung and black-eye beans (I). I selected beans with 1, 3 or 5 eggs on them. Beetles were allowed to emerge from beans as adults. I observed the effect of low larval competition (3 eggs / bean) and high larval competition (5 eggs /bean) on some life-history traits, i.e. development time, emergence mass and longevity. I studied also the effect of the host species and larval competition on the sex ratio of emerged adults (I).

2.4   Procedures for mating experiments

To studies II, III and IV, I collected beans with a single egg from my laboratory population. By using beans with only one egg, larval competition could be avoided inside the bean (Colegrave 1993, Horng 1997). Each single-egg bean was labelled and placed individually into a 1.5 ml Eppendorf tube. When the beetle emerged, the bean was removed from the tube. Pronotum width was used as a measure of body size. Eppendorf tubes were pierced to ensure supply of air.

Beetles were removed from their own tubes only when they were mated. During a mating a male and female were placed to the same Eppendorf tube. Duration of copulation was measured from the moment of penetration to the moment when the couple was separated from each other. The couple was observed during the whole copulation. After copulation they were replaced to their Eppendorf tubes.

In studies III and IV beetles were mated in a half-sib breeding design (Figure 1) where one male was mated to 3 (experiment 1 of study III) or 4 females (experiment 2 of study III and study IV) in two generations. When there were four females in P-generation, offspring was taken to experiment from only the three most productive ones. In studies II, III and IV male progeny was divided to control (non-mated) and mating group (3-4 times mated).

In ejaculate experiment (IV) the ejaculate was measured by weighing the male before and after copulation. The loss of weight was interpreted as ejaculate weight (Savalli & Fox 1998, Savalli & Fox 1999, Savalli et al. 2000).
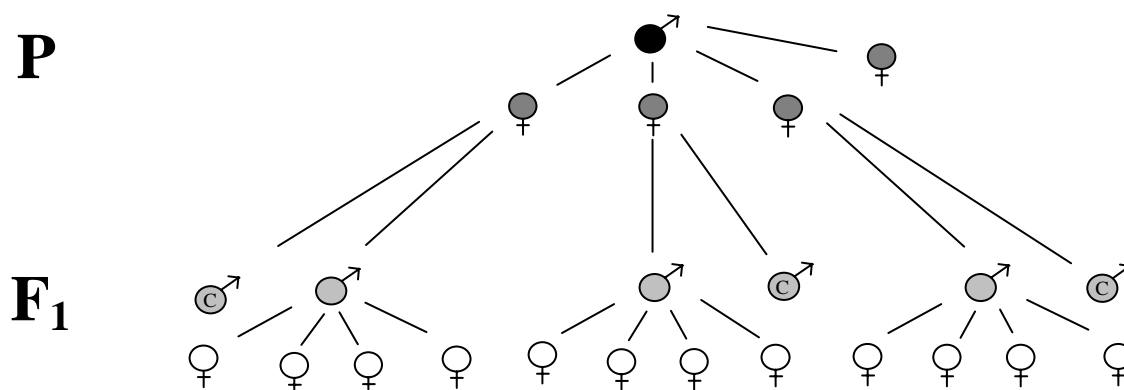

FIGURE 1   Half-sib breeding design of studies III and IV (Black = male parent, dark grey = female parent, light grey= male offspring, white = female partners of male offspring, c = control male). In study III, experiment 1 there were only three females for each male, and no females were excluded in P-generation.

3 RESULTS AND DISCUSSION

3.1 Larval competition and cost of mother's egg-laying decisions on offspring

In this study, females laid more eggs on black-eye beans than on mung beans (I). Earlier choice experiments between bean species have shown that *C. maculatus* usually prefers larger beans within bean species (e.g. Cope & Fox 2003) as well as between bean species (Kawecki & Mery 2003, but see also Messina 2004). Host preference and population differences may have some influence on a female's decisions (Kawecki & Mery 2003, Messina 2004). However, when I calculated this data per unit surface area, the number of eggs per surface area was not higher in black-eye beans. Moreover, these results showed that females laid their eggs at random on beans rather than distributing them evenly across the available beans.

In evolution of reproductive decisions in this species, intense larval competition (e.g. Colegrave 1993) or variance in the suitability of the larval food resource could be among causing factors. The latter could be of some importance in *C. maculatus*, since these beetles collect all energy they need during their larval stage (e.g. Fox & Tatar 1994). Furthermore, the quality of nutrition may have influence, as black-eye beans contain more energy and fat than mung beans (http://www.reformi-keskus.com/binary.aspx?sectionID= 2829&documentID=2428). However, the volume of black-eye beans is also about five times larger than that of mung beans, and thus there are more to eat for larvae.

In mung beans the emergence mass was decreasing with increasing larval competition but in black-eye beans larval competition did not affect the emergence mass of beetles. Pronotum width followed the same pattern. Development time was generally longer for larvae that developed in black-eye beans, and there was a clear sex effect: females needed longer development time than males. Longevity of individuals decreased due to larval competition in smaller bean species (mung bean) but not in larger one (black-eye bean).

However, the effect of development time on fitness is not clear, when development time did not affect longevity. I have also observed in my population that if there are very many eggs laid in one bean, larval competition becomes forceful, and larvae may emerge outside the bean before they are adults, and die (Paukku & Kotiaho, personal observation). Furthermore, since the adult life of *C. maculatus* consists mainly of finding a mate and performing reproductive functions, it is logical to think that with decreasing energy reserves fecundity decreases. Previous work has shown that larval competition has no direct effect on adult fecundity (Colegrave 1993), but an indirect effect in beetles raised on mung beans through lowered emergence weight and thus lowered fecundity, as emergence weight and fecundity correlate positively. However, I assume that even an indirect effect on fecundity is enough for avoidance of larval competition to evolve. Interestingly, the effect of emergence mass to longevity increased with increasing larval competition.

For a given size, females gain more mass in black-eye beans than in mung beans while there is no such difference in males. When there is a difference in selection to body size, it can be assumed that females allocate sex of their offspring to maximize their fitness. Beetles raised singly in smaller mung beans develop as large as singly raised beetles in bigger black-eye beans, but if 2 or even 3 larvae have to compete with each other, they will develop as smaller adults. This is why females could first lay female eggs when females have higher fecundity, and later male eggs, when even small males can copulate. Thus sex ratio could be at first female biased but later male biased or 50:50. I analysed the sex-ratio of the emerged beetles and interestingly found a strong bias towards females in mung beans that had only 1 egg on them. However, proportion of sexes was equal in larval competition situations or in black-eye beans with 1 egg. However, as the sex bias result in the larval competition experiment was not a pre-planned analysis, I conducted a formal experiment to verify the result. Although the results were not significant, they tended to be in the same direction. i.e. that females laid more female eggs on unoccupied mung beans.

3.2   Reproductive trade-off between copulation and longevity

There was a cost of reproduction seen as reduced longevity, when non-mated males lived longer than three-time mated males (II). I expect that ejaculate formation could be the cause of the cost. This is because males lose between 5–10% of their body mass in their first ejaculate (Fox et al. 1995, Savalli & Fox 1999, study IV). When studying the cost of reproduction it seems that many factors must be taken into consideration before general conclusions about the exact nature of the costs can be made.

Male mating status had an effect on the duration of copulation; the third copulation of the male lasted longer than the first or second, but there was no

difference between the first and the second copulation. Furthermore, the female that was the third mate of the male lived a shorter time than the first or the second mate, but there was no difference between longevities of the first two mates. Male identity had also an effect, but the duration of copulation had no effect on the longevity of his mate. Male mating status or male identity had no effect on their mate's egg production. However, there was a positive correlation between male body size and the number of eggs produced by mates of the male as well as between male body size and the duration of copulation.

It seems that male mating status has an effect on the longevity of his mate only if the female is allowed to lay eggs. This discrepancy suggests that the difference in female longevity may not be simply due to a beneficial increase in energy reserves of the first two mates of a male, as suggested above, but rather, that obtaining additional energy may increase the tolerance of the cost of reproduction of the first two mates of the male in comparison to the third mate.

3.3   Quantitative genetics of reproductive trade-off

I found a significant genetic variation in the tolerating the cost of reproduction (III). In other words, the realised absolute cost of unit reproductive effort varies between genotypes creating possibilities for selection to act on this trait closely related to fitness.

The genetic correlations were high between longevity and pronotum width under both of the mating treatments, especially in experiment three, suggesting that the tolerance of the cost of mating is to a considerable extent dependent on the resource gathering ability of the beetle larvae. In the same line, positive genetic correlations between body size and other aspects of life history have been documented in *C. maculatus* (Fox 1994).

This data set also showed that genotypes that survived longest in the control treatment groups also survived a relatively long time when they experienced a high mating rate. This was revealed by the positive rank-order correlation between the genotypes in the different environments, and also by the significant between-environment genetic correlation for longevity.

A classic example of negative pleiotropy is that adaptations to one environment leave the organism maladapted to the other (Lynch & Walsh 1998). According to this, in this study there should be negative between environment genetic correlations. However, as this was not what I found, I still could speculate that if environmental conditions were favourable, there could be negative correlations. Mutation-selection balance can explain some significant interactions in costly traits. In this data, mutations could explain the fact that some genotypes live long with or without reproduction.

A question remains, what causes the genetic variation in the tolerance of the cost of reproduction. In *C. maculatus* males invest heavily on the ejaculates: males lose up to 10% of their body mass in their first ejaculate (Fox et al. 1995,

Savalli and Fox 1999, study IV). Thus, depending on what the ejaculate is composed of, males invest either a large amount of limited energy reserves or water in their ejaculates.

Further studies (IV) indicate that there is genetic variation in the ejaculate size, not just as a dam effect (Savalli & Fox 1998), but also as a sire effect. According to this, males of some genotypes produce larger ejaculates than males with some other genotypes. The available evidence from other studies seems to indicate that sperm traits have a heritable genetic basis (for a review see Simmons 2001, and Simmons & Kotiaho 2002). Furthermore, the ejaculate size had an effect on male longevity giving expectations that this ejaculate size could explain the result of study III.

Further analysis showed that those genotypes that live longer time without reproduction have larger ejaculates and thus, the higher cost of reproduction. Because of this, I can conclude that there are differences between genotypes in investing for reproduction. As the cost of reproduction was larger in families that had larger ejaculate sizes, I can state that the ejaculate size is the most probably the explaining factor for the cost of reproduction in *C. maculatus* male seed beetles.

4 CONCLUSIONS

Even though the cost of reproduction as reduced longevity has not been previously reported for male *C. maculatus*, cost of reproduction is often documented in female *C. maculatus*. Generally, the cost of reproduction for males has been studied much less than that in females. Historically, it was assumed that sire invests in offspring only by copulating and donating sperm with its genes (see Simmons 2001). However, now it is recognized that investing resources into offspring may be an important strategy also for males (Wedell & Karlsson 2003). There is also growing evidence that male reproductive effort is more complex than has traditionally been thought.

Here I have shown that female's egg laying decisions may have an effect on offspring fitness. In other words, larval competition or quality of nutrition may give a cost that can be seen as reduced adult body size and emergence mass, or longevity (I). More importantly, I studied the effect of male reproductive investments on a fitness related trait. I found that there was a cost of reproduction, seen as reduced longevity (II). Furthermore, there was genetic variation in tolerating the cost of reproduction (III). Thus, I conclude that *C. maculatus* seed beetle males must trade-off their longevity with reproduction, and that there is genetic variance in this trade-off. Moreover, I showed how there is genetic variation in both the ejaculate size and in the tolerance of cost of reproduction (IV). I showed also that those genotypes that live longer time without reproduction have larger ejaculates and thus, the higher cost of reproduction. According to this, I can conclude that there are differences between genotypes in investing for reproduction. As the cost of reproduction was larger in families that had larger ejaculate sizes, I can state that the ejaculate size is the most probable explaining factor for the cost of reproduction. Nonetheless, an ejaculate is a major investment for a male, especially when energy reserves are limited.

These results suggest that not only the variance in reproductive effort but also the variance in tolerating the cost of that effort needs to be taken into account before full comprehension of the nature of the trade-offs between reproduction and other fitness related traits can be achieved. This study confirms how reproductive investments of males are far more complex than just a copulation and cheap sperm.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Lisääntymisen kustannukset jyväkuoriaisella – kvantitatiivisen genetiikan näkökulma

Lisääntymisestä aiheutuu melkein aina kustannuksia yksilölle. Tämä on nähtävissä vaihtokauppatilanteena lisääntymisen ja jonkin kelpoisuuteen vaikuttavan tekijän välillä. Yksi tällainen vaihtokauppatilanne muodostuu lisääntymisen ja elinajan välille. Evoluution ehtona tällaisessa tilanteessa on geneettinen vaihtelu lisääntymisen kustannusten sietokyvyssä.

Myös jälkeläisille voi muodostua kustannuksia vanhempien lisääntymispäätöksistä. Aloitin väitöskirjatyöni tarkastelemalla naaraan munintapäätösten vaikutusta sen jälkeläisten kelpoisuuteen vaikuttaviin ominaisuuksiin. Tutkimuslajinani oli jyväkuoriainen *Callosobruchus maculatus*. Tämä trooppinen tuholainen soveltuu hyvin kustannustutkimuksiin, sillä kuoriaiset keräävät kaiken aikuisena tarvitsemansa energian toukka-aikanaan, ja täten selviävät ja pystyvät lisääntymään ilman ruokintaa. Tutkimuksissa ilmeni, että kuoriaisnaaras muni enemmän munia mustasilmäpavuille kuin mungpavuille. Jos kuitenkin huomioitiin papujen pinta-ala, niin pinta-alayksikköä kohti laskettuna munien määrässä ei ollut eroa papulajien välillä. Tämä osoittaa, ettei naaras valikoi papujen välillä, vaan muninta niille on satunnaista. Toukkakilpailun huomattiin kuitenkin aiheuttavan jälkeläisille kustannuksia, jotka ilmenivät alentuneena ruumiinkokona tai yksilön massana kuoriutumishetkellä, ja/tai lyhentyneenä elinikänä.

Väitöskirjatyöni pääasiallisena tutkimuskohteena olivat lisääntymisen kustannukset koirailla. Tutkimuksissa selvisi, että koiraille muodostuu lisääntymisestä kustannuksia, jotka ilmenevat elinajan lyhenemisenä paritelleilla koirailla. Lisäksi halusin selvittää, onko lisääntymisen kustannusten sietokyvyssä geneettistä vaihtelua. Tätä kysymystä tutkin kvantitatiivisen genetiikan menetelmillä. Havaitsin, että lisääntymisen kustannusten sietokyvyssä oli vaihtelua genotyyppien välillä.

Tutkimuslajillani ejakulaatti on suuri panostus, varsinkin kun koiraalla on vain rajallinen määrä energiaa käytettävissään aikuisiällään. Ensimmäisen ejakulaatin massa voi olla jopa 5-10 % kuoriaiskoiraan massasta. Tästä johtuen halusin selvittää, voisiko geneettinen vaihtelu ejakulaatin koossa olla selityksenä geneettiselle vaihtelulle lisääntymisen kustannuksessa. Tutkimuksissa ilmeni, paitsi että ejakulaatin koossa oli geneettistä vaihtelua, myös ejakulaatin koko vaikutti elinikään. Lisäksi niillä genotyypeillä, jotka elivät pitkään ilman lisääntymistä, oli lisääntyessään suurin ejakulaatin koko, ja siitä johtuen myös suurin lisääntymisen kustannus. Väitöskirjatyöni perusteella voidaan sanoa, että koiraan lisääntymispanostus on paljon moniselitteisempi asia, kuin perinteisesti on ajateltu.

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