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Reproductive strategies of *Tribolium* flour beetles

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Abstract

Although, beetles of the genus *Tribolium* first evolved as saprophylic insects, they have adapted to the stored products environment for several thousand years. In this study reproductive strategies are described for eight species of *Tribolium* that are known to occur in this environment. Experiments were conducted under the same conditions for every species, and several life history traits, including egg mass, adult mass, developmental time and fecundity were examined and compared among these species. Common reproductive strategies were not found among the different species and univariate analysis highlighted strong differences between the species for most of the traits investigated. Some species showed reproductive traits that are likely to give a fitness advantage in the environment of stored products. Multivariate statistical analysis allowed the detection of different sub-groups with respect to their reproductive strategy. Adult mass and egg-to-adult developmental time discriminated between groups. Intraspecific allometric relationships were further investigated but only a few correlations appeared to be significant.

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Introduction

In oviparous animals, egg size is a particularly important life history trait because it affects both maternal and offspring fitness (Fleming and Gross, 1990) including initial offspring resources and size. An individual's resources can be allocated to three basic functions: growth, somatic maintenance, and reproduction. One assumption of life history theory is that limiting resources lead to trade-offs between different structures and activities. In a phenotypic trade-off, there is differential allocation of resources between traits, which means that an individual that devotes more resources to support one trait will have fewer resources to invest in another (Berrigan, 1991; Bernardo, 1996; Fleming and Gross, 1990; Messina and Fox, 2001; Smith and Fretwell, 1974). Thus, for a fixed amount of resources available for reproduction, it necessarily follows that there is a trade-off between the number and size of offspring during a given bout of reproduction (Bernardo, 1996; Messina and Fox, 2001), resulting in an egg size that is a balance between selection for large eggs and selection for many eggs. In addition, egg size and egg number are generally correlated to body size which have important ecological and evolutionary implications for fitness (Fleming and Gross, 1990). Also, developmental time depends on both egg and adult body size with progeny developing from larger eggs growing generally faster, attaining larger size, and having higher survivorship than progeny developing from smaller eggs (Fleming and Gross, 1990; Fox, 1994a and Fox, 1994b). These predictions were tested here using beetles of the genus *Tribolium*.

Iteroparous beetles of the genus *Tribolium* McLay (1825) (Coleoptera, Tenebrionidae) have been associated with stored food for more than 4000 years (Levinson and Levinson, 1985), equivalent to about 30,000 generations of *Tribolium castaneum* and *Tribolium confusum*, the most common flour beetles (Sokoloff, 1972). Together with other pests of stored products (Levinson and Levinson, 1994, 1995), *Tribolium*, however, primarily evolved as saprophylic insects and naturally occur under the bark of trees, in dead wood and occasionally in the nests of some Hymenoptera (Grimm, 2001; Halstead, 1969; Magis, 1954; Sokoloff, 1974).

In the present work, several life history traits were compared, including egg size and number, developmental time, nymph and adult mass among eight *Tribolium* species. In order to determine if similar reproductive strategies are adopted by the different species under investigation, multivariate analysis were performed. In addition, intraspecific allometric relationships were established.

Materials and Methods

Study species

Eight species of flour beetles were examined (Table 1). Most of the populations studied here have been cultured under laboratory conditions for 20 years or more before the start of the experiments. They were obtained from several laboratories where they had been cultured under similar rearing conditions such as diet, temperature and relative humidity. Moreover, they were cultured for at least one year (i.e. about seven generations of the slowest species, *T. brevicornis*, see results) in our laboratory under the same conditions before the start of the study.

In addition to the different species investigated, two strains of both *T. castaneum* and *T. confusum* that differed significantly in body size were included in the experiments. The *T. castaneum* strains were originally derived from a single population and were selected during 400 generations for body size (Bartlett et al., 1966). These two strains were used to explore the effect of selection for body size on life history traits. The two

Table 1. *Tribolium* species and origin of the laboratory strains used in the study.

Species	Common name	Origin and year of collection	
		Country	Year
T. anaphe (Hinton)	-	Nigeria	1956
T. audax Halstead	American flour beetle	Canada	1969
T. brevicornis (LeConte)	Giant flour beetle	USA	unknown
T. castaneum (Herbst) "large"	Red flour beetle	Purdue Univ., USA	1954
T. castaneum (Herbst) "small"			
T. confusum DuVal "Africa"	Confused flour beetle	Guinea Konakry	1996
T. confusum DuVal "Germany"		Germany	1967
T. destructor Uyttenboogaart	Dark flour beetle	Ethiopia	1968
T. freemani (Hinton)	Kashmir flour beetle	Japan	1980
T. madens (Charpentier)	Black flour beetle	Yugoslavia	1959

T. confusum populations had different geographic origin and had most probably encountered different selective histories. The small individuals were from a German population (Berlin, Germany) and had been cultured under laboratory conditions for more than 30 years, whereas the large individuals originated from an African population (Dalaba, Guinea-Conakry) collected in the field three years before the start of the experiments.

Comparative studies are often made on data taken from the literature (Berrigan, 1991; Garcia-Barros, 2000; Holloway et al., 1987; Honek, 1993; Stewart et al., 1991), but conditions of culture, especially temperature and food, and genetic origin of the organisms can affect their reproductive traits. Intra- and inter-specific life history variations may therefore result from different biotic and abiotic environmental conditions instead of true species-specific differences (Vanderpoorten and Jacquemart, 2004). To eliminate this source of variation, we collected our own data and measured these traits keeping all species under the same rearing conditions beetles, in a dark incubator at 25 ± 2°C and 65 ± 5% relative humidity with wheat flour and brewer's yeast (10/1 w/w) as rearing medium. Although it would have been ideal to examine these reproductive traits under a range of conditions such as different food, relative humidity and temperature, these rearing conditions were chosen because they allowed the proper development of every species.

Life history traits

The relationships between female size, egg size and fecundity were examined first. The relationships between egg size and different offspring characteristics, e.g. adult mass and developmental time were also measured. The fresh mass of the eggs, nymphs and adults was used as an index of size. Eggs, nymphs and adults were weighed to the nearest 10^{-4} mg (Sartorius super-micro balance, Sartorius Instruments, www.sartorius.com).

Female mass versus egg characteristics. Twenty females were placed with 20 males in a 90 mm petri dish with 20 g of rearing medium. Males were marked with a black pencil to facilitate their identification. After five days, the males were discarded and the females placed individually in a 55 mm petri dish with 5 g of rearing medium. The number of eggs laid by each female was recorded every second day during six days. Depending on female fecundity, up to a maximum of 15 eggs were weighed for every female (five eggs for each egg laying period). Females were weighed at the end of the experiment. All adults were virgin at the start of the experiment and were 30-35 days post-eclosion.

Egg mass versus offspring characteristics. For each species, the following offspring characteristics were examined: egg (E_M), nymph (N_M) and adult (A_M) mass and egg-to-nymph (E-N D_T), nymph-to-adult (N-A D_T) and total developmental time (E-A D_T). One hundred unsexed beetles of each species were placed in about 100 g of rearing medium for 24 h. After that period, the eggs laid were collected and 75 eggs were weighed. Eggs were placed individually in a glass tube with about 1g of rearing medium. After three weeks, the tube was examined daily for the presence of nymphs. On the day after pupation, nymphal sex and mass were determined. The nymphs were kept individually until adult emergence. Adults were weighed on the day after their emergence. Due to the mortality of eggs, larvae and nymphs during the experiment, sample size was neither constant nor equal to 75 in every species. Indeed, preliminary experiments showed that the manipulation of the eggs and the nymphs had no affect on the survivorship of the individuals.

Statistical analyses

Interspecific comparisons: ANOVA, PCA and clustering. Analysis of variance (ANOVA) and subsequent Tukey multiple comparison tests were carried out to compare the different reproductive traits of the species studied, using the SAS system V8.02 (SAS Institute, 2001). A sequential Bonferroni test was used to control for type I error within groups where the same variable was tested (Rice, 1989). In animals, a size dimorphism is often observed between males and females (Fairbairn, 1996), and it is important to consider sexual difference in life-history trait studies (Fischer and Fiedler, 2001a; Guntrip et al., 1997). In insects, females are often larger than males. Adult size dimorphism was tested within the different species using one-way ANOVA. In addition, one-way ANOVAs were conducted to compare egg mass and developmental time between each sex within every species/population. Further analyses were performed with the R statistical software version 1.4.1 (Ihaka and Gentleman, 1996). A principal component analysis (PCA) of the developmental traits was carried out to highlight the similarities and differences of reproductive biology between the species examined. The PCA was achieved on a correlation matrix and based on rotated principal components. In addition, a hierarchical cluster analysis was implemented using Ward's method (Ward, 1963) on the matrix of Euclidean distances between the species, calculated from the standardized specific $\log_{10}$ means of the observed reproductive traits. Throughout the text, all means are presented ± 1 SEM.

Intraspecific allometry. To examine how offspring characteristics scale with each other, Pearson correlation coefficients were calculated and associated least squares linear models. Data were $\log_{10}$ transformed prior to the analysis to ensure homoscedasticity of the variables across the species, given the observed relations between $\log_{10}$ specific variances and means (LaBarbera, 1989). Several allometric relationships were tested and, where necessary, controlled for prior to analysis of the residuals.

Results

Between species/strains comparisons: ANOVA, PCA and clustering

Egg mass and female fecundity. Results are presented in Table 2. Egg mass, female mass and female fecundity differed significantly between the *Tribolium* species (one-way ANOVA, $F_{9,183}=91.0$, $P<0.01$; $F_{9,190}=107.9$, $P<0.001$; $F_{9,183}=32.8$, $P<0.001$, respectively). The Tukey multiple comparison test determined similar differences between the species as found in the next experiment for egg and adult mass (see Table 3). However, because of the smaller sample size relative to the second experiment, the differences of adult mass between the strains were less strong (compare Tables 2 and 3). *Tribolium castaneum* females from the "large" strain and *T. destructor* females were the more and the less fecund, respectively. The fecundity observed here for *T. destructor* was very weak compared to the data from the literature (e.g. Reynolds, 1944) and only 13 females laid over 20 eggs (with the fecundity of only three females ranging from 18 to 39 eggs and from 1 to 5 eggs in the remaining 14). Examining the results of the two *T. castaneum* strains, an effect of female size on fecundity was observed, with the females of the "small" strain being less

Table 2. Egg mass, female fecundity and female mass in eight *Tribolium* species. Female fecundity was observed during three oviposition period of two days.

Species	Egg mass^{1,2}		Female mass^{1,2}		Female fecundity^{1,3}	
T. anaphe	0.0615 ± 7.3 10-4 (20)	de	4.296 ± 0.124 (20)	cd	37.6 ± 2.9 (20)	cd
T. audax	0.0975 ± 10.8 10-4 (20)	a	2.689 ± 0.048 (20)	e	19.9 ± 1.3 (20)	ef
T. brevicornis	0.0948 ± 18.8 10-4 (20)	ab	7.951 ± 0.533 (20)	a	25.8 ± 2.2 (20)	de
T. castaneum "large"	0.0491 ± 10.7 10-4 (20)	f	2.681 ± 0.048 (20)	e	87.8 ± 8.3 (20)	a
T. castaneum "small"	0.0412 ± 6.3 10-4 (20)	g	0.890 ± 0.019 (20)	f	36.7 ± 1.7 (20)	cde
T. confusum "Africa"	0.0641 ± 8.2 10-4 (20)	d	3.949 ± 0.134 (20)	d	63.7 ± 4.9 (20)	b
T. confusum "Germany"	0.0561 ± 8.6 10-4 (20)	e	2.405 ± 0.050 (20)	e	50.6 ± 3.8 (20)	bc
T. destructor	0.0737 ± 16.6 10-4 (13)	c	5.427 ± 0.109 (13)	b	8.0 ± 3.2 (13)	f
T. freemani	0.0888 ± 11.6 10-4 (20)	b	5.036 ± 0.159 (20)	bc	33.5 ± 3.3 (20)	cde
T. madens	0.0783 ± 7.2 10-4 (20)	c	5.039 ± 0.083 (20)	bc	28.8 ± 2.1 (20)	de

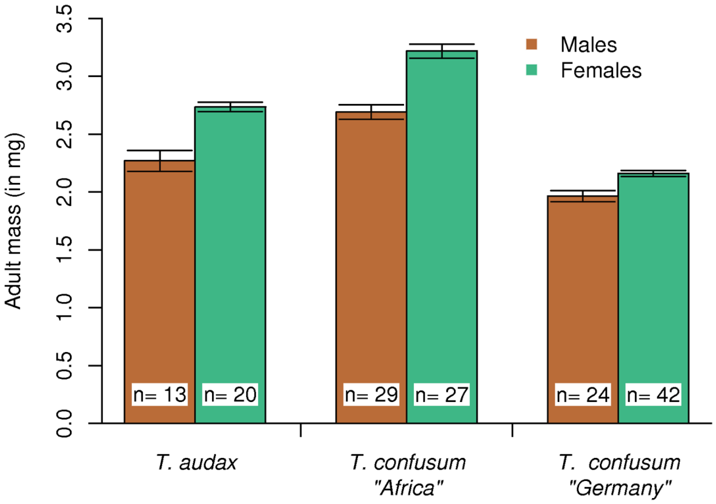
1: mean ± se (sample size), 2: in mg, 3: number of eggs laid during 6 days. In the same column, the data followed by the same letter are not statistically different (Tukey test, P>0.05).

Table 3. Egg, nymph and adult mass of eight *Tribolium* species.

Species	Mass^{1}					
	Egg		Nymph		Adult	
T. anaphe	0.0698 ± 10.5 10-4 (75)	e	4.599 ± 0.052 (60)	e	3.826 ± 0.051 (55)	d
T. audax	0.0917 ± 15.9 10-4 (75)	bc	2.967 ± 0.064 (37)	g	2.552 ± 0.058 (33)	f
T. brevicornis	0.0956 ± 12.7 10-4 (75)	a	12.960 ± 0.231 (55)	a	10.983 ± 0.191 (53)	a
T. castaneum "large"	0.0517 ± 75.0 10-5 (75)	g	2.983 ± 0.067 (60)	g	2.404 ± 0.036 (55)	f
T. castaneum "small"	0.0454 ± 70.0 10-5 (75)	h	0.796 ± 0.020 (58)	i	0.666 ± 0.017 (54)	h
T. confusum "Africa"	0.0695 ± 13.1 10-4 (75)	e	3.538 ± 0.072 (59)	f	2.945 ± 0.056 (56)	e
T. confusum "Germany"	0.0585 ± 92.0 10-5 (75)	f	2.628 ± 0.288 (68)	h	2.097 ± 0.026 (66)	g
T. destructor	0.0886 ± 12.7 10-4 (75)	c	6.280 ± 0.129 (21)	b	5.299 ± 0.113 (21)	b
T. freemani	0.0920 ± 13.5 10-4 (75)	b	5.794 ± 0.056 (62)	c	5.089 ± 0.049 (61)	b
T. madens	0.0799 ± 11.8 10-4 (75)	d	4.998 ± 0.045 (56)	d	4.300 ± 0.043 (56)	c

1: mean ± se (sample size), in mg. In the same column, the data followed by the same letter are not statistically different (Tukey test, P>0.05).

Fig 1. Mortality of uziflies in tetracycline treated and control batches.



fecund than females of the "large" strain, despite the fact that they produce smaller eggs (Table 2).

Egg mass and offspring characteristics. *(i) Male/female differences*. Significant differences between adult male and female mass were only found in *T. confusum* "Africa" and "Germany" and in *T. audax* (one-way ANOVA, $F_{1,54}$= 36.6, P<0.001; $F_{1,64}$= 16.0, P=0.001; $F_{1,31}$= 27.9, P<0.001, after sequential Bonferroni correction, respectively – Figure 1). Neither egg mass nor developmental time differed significantly between males and females in any species (one-way ANOVA, $F_{1,58}$=6.3, P_{Min}=0.152; $F_{1,66}$=4.0, P_{Min}=0.488, after sequential Bonferroni correction, respectively). Therefore, subsequent analyses were conducted with the data pooled across males and females. *(ii) Interspecific comparisons*. Egg mass, nymph mass and adult mass were significantly different between the different species (one-way ANOVA, $F_{9,740}$=239.0, P<0.001; $F_{9,526}$=1375.9, P<0.001; $F_{9,502}$=1429.3, P<0.001, respectively - Table 3). Egg-to-nymph, nymph-to-adult and total developmental time did also differ significantly between species (one-way ANOVA, $F_{9,526}$=270.7, P<0.001; $F_{9,508}$=104.8, P<0.001; $F_{9,508}$=368.1, P<0.001, respectively - Table 4). A Tukey multiple comparisons' analysis was thus performed to compare the species. The groups obtained are presented in Tables 3 and 4. *Tribolium brevicornis* and *T. castaneum* "small" had the biggest and the smallest egg and adult mass, respectively. In parallel, these species had the quickest and the smallest developmental time, respectively. Although *T. audax* adults were among the smallest and possessed eggs as big as *T. brevicornis* did, their total developmental time was one of the longest. However, time to emergence as an adult (nymph-to-adult developmental time) was the shortest in *T. audax*.

Within *T. castaneum*, beetles from the "large" strain were, on average, 3.5 times heavier than adults from the "small" strain (Table 3). Although egg size and total developmental time were also different between these two strains (Tables 3 and 4), a similar difference was not observed (E_M ratio=1.14, D_T ratio = 1.08).

1: mean ± se (sample size), in days. In the same column, the data followed by the same letter are not statistically different (Tukey test, P>0.05).

To examine which trait had the most influence on the determination of the developmental characteristics of the *Tribolium* species studied here, a principal component analysis was performed using the average values of the inter-specific data ($\log_{10}$ mean value of every trait for every species/population). The principal

Table 4. Egg to nymph, nymph to adult and total (egg to adult) development time (in days) for eight *Tribolium* species.

Species	Egg to nymph		Nymph to adult		Egg to adult (total)	
T. anaphe	31.1 ± 0.3 (60)	d	7.3 ± 0.1 (55)	cd	38.1 ± 0.2 (55)	e
T. audax	40.8 ± 0.9 (37)	a	6.1 ± 0.1 (34)	f	46.2 ± 0.7 (34)	b
T. brevicornis	40.1 ± 0.5 (55)	a	9.1 ± 0.1 (53)	a	49.1 ± 0.6 (53)	a
T. castaneum "large"	25.3 ± 0.3 (60)	f	6.6 ± 0.1 (57)	e	31.7 ± 0.3 (57)	h
T. castaneum "small"	23.1 ± 0.3 (58)	g	6.3 ± 0.1 (55)	f	29.3 ± 0.3 (55)	i
T. confusum "Africa"	29.5 ± 0.3 (59)	e	7.1 ± 0.1 (56)	d	36.5 ± 0.3 (56)	f
T. confusum "Germany"	25.6 ± 0.2 (68)	f	7.3 ± 0.1 (68)	c	32.9 ± 0.2 (68)	g
T. destructor	32.4 ± 0.5 (21)	c	8.0 ± 0.1 (21)	b	40.4 ± 0.4 (21)	d
T. freemani	34.4 ± 0.2 (62)	b	7.4 ± 0.1 (61)	c	41.8 ± 0.2 (61)	c
T. madens	30.6 ± 0.2 (56)	d	7.1 ± 0.1 (56)	d	37.7 ± 0.2 (56)	e

component analysis gives a good understanding of the multidimensional relationships between the traits (Figure 2). The first two components explained more than 95% of the initial variability, with 81.6 and 15.0% of the variance explained by the first and the second principal component, respectively. The two last components contributed to only 2.4 and 1.0% of the variance, respectively. The first axis (PC1) is a scale axis where we can distinguish the larger species with longer developmental time from smaller ones with shorter developmental time. The second axis (PC2) refines this distinction with species of the same size being separated from each other upon egg-to-nymph developmental time.

The visual interpretation made on the principal component analysis was completed by a cluster analysis. Interesting convergences, which are shown in a dendrogram, were detected (Figure 3). Each node represents the levels of similarity between the different species/populations merged on the basis of the traits measured. Similarity is defined as one minus the ratio of the estimated distance between the merging clusters on the maximum value of the original distance matrix. The dendrogram is cut before the breakpoint in the slope of the decreasing similarity level during the clustering process, defining six groups that are easily identifiable on the principal component analysis graphical representation (groups A to F -

Fig 2. Mortality of uziflies in tetracycline treated and control batches.

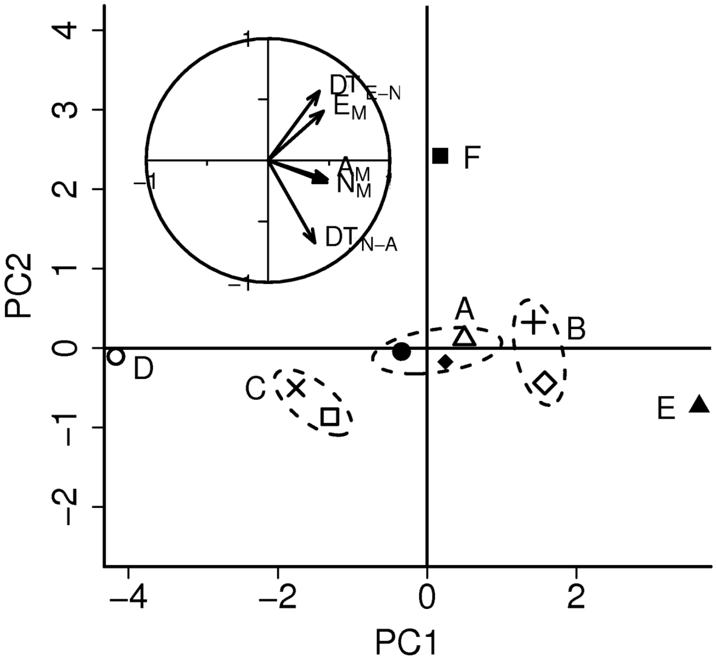
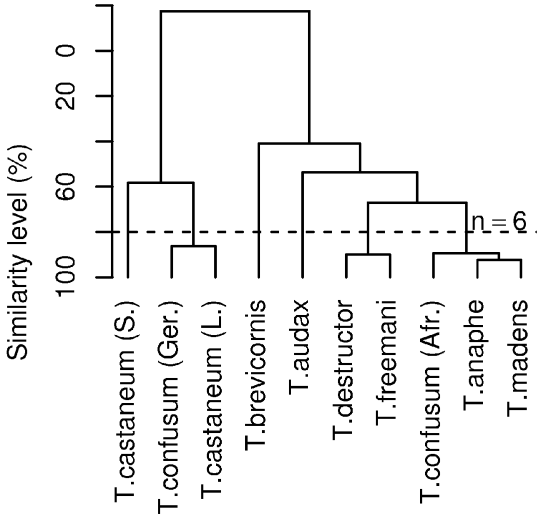


Figure 2) and shares a final similarity higher than 85%.

Fig 3. Mortality of uziflies in tetracycline treated and control batches.



Intraspecific allometry

Egg mass and female fecundity. Three correlations were tested: egg mass vs. female mass, female fecundity vs. female mass and egg mass vs. fecundity. When significant correlation was found between female mass and fecundity, and/or between egg mass and fecundity, we controlled for these allometric relationships and calculated the residuals from the mean regression lines to examine the relationship between egg mass and number. Although between females variability for egg mass and number was significant within the species (two-way nested ANOVA, $F_{183,2296}$= 9.81, P<0.001, and $F_{190,400}$= 3.36, P<0.001, respectively - ANOVA were conducted on the basis of the observations made during the three egg laying periods), intra-specific relationships between female mass *versus* egg mass or number were mostly not found, and only one intra-specific correlation was significant after Bonferroni sequential correction (female mass vs. fecundity in *T. confusum* "Africa", Pearson test, r_{19} = 0.640, P = 0.020). The other intra-specific relationship, i.e. egg mass vs. fecundity, was not significant in any of the species/populations examined.

Egg mass and offspring characteristics. Nine correlations were tested between the traits under consideration (Table 5). Except for the relationship between nymph and adult mass, which was expected, most of the intra-specific correlations studied were not significant. Significant correlations (after sequential Bonferroni correction) were highlighted between adult mass and egg-to-adult developmental time, but their direction was opposite depending on the species: positive in *T. castaneum* "large" and "small" (Pearson test, r_{53} = 0.542, P<0.001 and r_{52} = 0.758, P<0.001, respectively) and negative in *T. destructor* and *T. freemani* (Pearson test, r_{19} = -0.763, P<0.001 and r_{59} = -0.456, P = 0.002, respectively). A similar phenomenon was detected for the correlation between nymph mass and egg-to-nymph developmental time: positive in *T. castaneum* "small" and *T. madens* (Pearson test, r_{56} = 0.772, P<0.001 and r_{54} = 0.379, P = 0.024, respectively) and negative in *T. anaphe*, *T. destructor* and *T. freemani* (Pearson test, r_{58} = -0.378, P = 0.020; r_{19} = -0.642, P = 0.014 and r_{60} = -0.455, P = 0.002, respectively).

Table 5. Allometric relationships tested across developmental traits.

Traits*	NM	AM	DTe-n	DTn-a	DTe-a
EM	X**	X	X**	-	X**
NM	-	X	X	X**	-
AM	-	-	-	X	X

*: EM: egg mass, NM: nymph mass, AM: adult mass, DTe-n: egg to nymph development time, DTn-a: nymph to adult development time, DTe-a: egg to adult (total) development time. **: Correlation were calculated on residuals from the regression of $\log_{10}$ "variable" on $\log_{10}$ nymph or adult mass.

Discussion

As concluded from previous theoretical and empirical studies, larger and smaller *Tribolium* species were found to adopt different reproductive strategies, and larger species did not invest in reproduction as much as smaller ones do, relative to their body size. Individuals may emerge as an adult in as short a period of time as possible. Thus, there is an optimal balance between egg size, fecundity and developmental time in every species and population of a species. Significant differences were detected for most traits and multivariate analysis emphasized different sub-groups for the reproductive strategies adopted by the species. Species such as *T. castaneum* and *T. confusum* presented reproductive traits that are likely to give them a reproductive advantage in the environment of stored products. These species may have occurred in this ecosystem for longer periods than the others. However, as no outgroup species was available, i.e. a species of *Tribolium* that still occurs in its natural habitat, the observed differences could also result from ancestral species differences rather than a response to adaptative changes, with some *Tribolium* species being naturally pre-adapted to this environment. Furthermore, we can conclude that smaller species do better in this environment than larger ones (but see *T. audax*).

Atypical developmental time was noticed for *T. audax*. Despite the fact that adults of that species are one of the smallest and possess eggs as big as those of *T. brevicornis* (the biggest species examined here), their total developmental time was one of the longest. However, time to emergence as an adult (nymph-to-adult developmental time) was the smallest in *T. audax*. Physiological constraints of the larval stage seem therefore to slow down their developmental time, which could reflect a recent adaptation to that environment. In *Tribolium*, cannibalism is an important factor of population growth regulation with the pupal stage being one of the most vulnerable (Park et al., 1965, 1970). The quick emergence of *T. audax* nymph may reduce their vulnerability at this stage. However, the advantage of producing larger eggs is clear in this species since the developmental time of *T. audax* individuals is one of the longest (Table 4). Producing many small eggs instead of a few large ones is likely to decrease female fitness because of increased offspring developmental time.

By investigating intraspecific variations of life history traits between populations showing significant differences in body size, i.e. *T. castaneum* "small" and "large" and *T. confusum* from Germany and Africa, interesting reproductive trade-offs were found. The strong selection applied on adult size of the "large"and "small" strains of *T. castaneum* has not lead to similarly strong selection on egg size and total developmental time. *Tribolium castaneum* "large" are about 3.5 times larger than adults of the "small" strain, but their egg and developmental time are only about 1.1 times higher. It is likely that there is a minimum egg size that is necessary to maintain egg viability. Also, a reduction in adult size does not lead to a severe diminution of egg-to-adult developmental time. Even if adults have been selected to be as small as possible, the complete developmental cycle requires a minimum time. In addition, selection of larger adults has not led to the production of larger eggs. The egg characteristic of each species may represent an optimum size at which viable larvae can be formed, which is balanced by the need to spread reproductive investment into the production of many eggs (Wilbur, 1977). Maximum egg size may also be limited by physical restrictions imposed by the structure of the ovipositor (Bernardo, 1996; Fitt, 1990). In spite of being one of the smallest species tested, *T. audax* females produced the biggest eggs (Tables 2 and 3), it seems therefore unlikely that the structure of the ovipositor is a limiting factor regarding the production of larger eggs in flour beetles.

Although *T. castaneum* females of the "small" strain produced smaller eggs than female from the "large" strain, they were not as fecund as females of the "large" strain. In other words, the decrease of egg mass did not compensate the selection made upon body size to enable a similar fecundity in the two strains (egg size of females from the "small" strain should have been reduced by about 2.5 in order to achieve a similar fecundity as females from the "large strain" - see results in Table 2). As predicted from developmental time data, a minimum offspring size seems necessary to maintain egg viability and/or to allow offspring to complete their development in an optimal time period. Otherwise, it should be expected that females of the "small" strain would produce more and smaller eggs than what we found. Similar tendencies were found between the two *T. confusum* populations. But, as these populations have different selective histories, the conclusions based on these results may be less certain. The "German" population has been cultured for more than 30 years in the same biotic and abiotic conditions whereas the "African" insects were collected in the field only three years before the experiment. The German population should be much more inbred than the African one, which can lead to reduced body size (Wade et al., 1996).

The principal component analysis and the cluster analysis (Figs. 2 and 3) emphasized different sub-groups of the reproductive strategies adopted by the species examined. Egg, nymph and adult mass and total developmental time appeared to discriminate between two groups of species (PC1). Larger species with longer developmental time are easily distinguished from smaller ones with shorter developmental time. The second axis (PC2) refines this distinction with species of the same size being separated from each other upon egg-to-nymph developmental time. The principal component analysis shows the atypical behaviour of *T. audax* according to this axis. Six groups of similarity (similarity higher than 85% within every group) are highlighted in the cluster analysis. Based on the multivariate statistical analysis, it appears that *T. audax* and *T. brevicornis* do not behave as it could be expected in regards to the results obtained with the other species. *T. audax* adults are smaller than expected with regard to egg mass, but they need a longer time to develop. *Tribolium brevicornis* adults are larger and their developmental time is also longer than expected regarding egg size, but in a less pronounced manner than in *T. audax*.

Although the two *T. castaneum* populations ("large" and "small") belong to the group of the "small" individuals, they belong to two distinct sub-groups, with the "large" strain having more common traits with *T. confusum* "Germany" than with the "small" strain. The selection for body size exerted on the parent population has driven to the selection of two highly distinct populations considering the reproductive traits under investigation. Only egg mass and developmental time were hardly affected by this selection. Similarly, in *Drosophila melanogaster*, selection on developmental time affects both adult body size and fecundity (Nunney, 1996). In addition, it is remarkable that the two *T. confusum* populations belong to much more distinct groups than the two *T. castaneum* populations and are probably genetically more variable.

Intraspecific allometry. There is widespread evidence of connections between reproductive traits (Berrigan, 1991; Ellers et al., 1998; Fleming and Gross, 1990; Fox and Czesak, 2000; Fox et al., 2001; Honek, 1993; Smith and Fretwell, 1974; Visser, 1994). Examining the triangular association between fecundity, egg size and adult body size, positive intra-specific correlations were found in some insects. However, the absence of correlation was frequently observed in others (Fitt, 1990; Fox and Czesak, 2000; Honek, 1993; Klingenberg and Spence, 1997). In flour beetles, we found a significant relationship between female mass and fecundity in only one species (i.e. *T. confusum* "Africa"), and the relation between female and egg mass was not significant in any species. Similarly, a very weak correlation between female mass and fecundity was reported in butterflies (Fischer et al., 2003). In addition, despite widespread reports of intra-specific trade-off between egg mass and number in Arthropods (Fischer and Fiedler, 2001b; Fox and Czesak, 2000 for a review; but see Bernardo, 1996), no significant relationships were found in any species. Indeed, the trade-off between size and number of progeny is generally observed in semelparous species (capital breeders – Stearns, 1992), but is more difficult to detect in iteroparous species (income breeders – Stearns, 1992) (see review by Fox and Czesak, 2000).

Both egg and adult sizes affect egg-to-adult developmental time. In bruchid beetles, for a given body size, adults from smaller eggs take longer to develop (Fox et al., 1999). Additionally, adults coming from larger eggs achieved a larger body size (Fox, 1994b, 1997). In butterflies, no relationships were found between egg size and developmental time (Wiklund and Persson, 1983) or between developmental time and body size (Karlsson and Wiklund, 1984, but see Fischer and Fiedler, 2002). Absence of relationship between developmental time and body size was also found in the stored-products beetle *Prostephanus truncatus* (Guntrip et al., 1997). In contrast, a positive correlation between developmental time and adult size was shown in *Gerris buenoi* (Klingenberg and Spence, 1997). Furthermore, it was discovered that the development period of female *P. truncatus*, but not of males, was negatively correlated to egg mass (Guntrip et al., 1997). Such a relationship was not determined in *Tribolium spp*. A positive correlation was found between egg mass and developmental time in the two *T. castaneum* populations investigated, but this correlation was negative in *T. destructor* and *T. freemani* . Significant relationships were also observed between nymph mass and egg-to-nymph developmental time in a few species. Other relationships were tested (see Table 5), but they were not significant.

Absence of significant intraspecific allometry is often reported in iteroparous organisms (Fox and Czesak, 2000) and interspecific allometry are commonly performed to show strongest relationships. As species are part of a hierarchically structured phylogeny related taxa cannot be regarded as independent data points for statistical purposes (Felsenstein, 1985; Pagel and Harvey, 1988). Pattern of association of some ecological characters could also be masked by a simple across species comparison (Harvey, 1996). Phylogenetic comparative methods have been proposed to account for phylogenetic independence. In addition, such comparative methods could give valuable information about the pattern of evolution of the different traits between the species (see Pagel, 1997; Freckleton et al., 2002 for reviews). In a separate study, the phylogenetic association between these species will be taken into account to establish interpecific allometric relationships, and to examine patterns of evolution of *Tribolium* reproductive characters.

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