

FITNESS OF FIVE PHENOTYPES OF *Harmonia axyridis* PALLAS (COLEOPTERA: COCCINELLIDAE).

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H. axyridis is a highly polymorphic ladybird beetle with phenotypes that vary in their elytral patterns. In natural populations the relative frequency of phenotypes is not constant, but is subject to a strong geographic and temporal variation. The selective forces that are the basis for those variation are diverse and difficult to determine. In this work we suggest that fitness of phenotypes could contribute to these variations. That is, a specific genotype could also confer a specific reproductive capacity. We compare the reproductive capacity of adults of five phenotypes [*succinea* (h) or s0, *succinea*-3 (h³) or s9, *conspicua* -1 (h^{C1}) or c1, *aulica*-1 (h^{A1}) or au and *nigra* [?(h[?])] or ni]. Our results show that under the same biotic and abiotic conditions, there are differences in biological parameters of the phenotypes that affect their fitness. The rare phenotype *nigra*, and the relatively uncommon *aulica* have lower reproductive capacity than the most frequent phenotypes *succinea* and *conspicua*.

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INTRODUCTION

Fitness is defined as a measure of the reproductive success of a genotype relative to another in the same environment. It measures the capacity of a genotype to transmit its genes to the next generation (BEGON et al. 1990). The direct evaluation of fitness has rarely been performed (HOFFMAN 1994). Generally, researchers measure fitness indirectly by using biological parameters such as viability (survival, fecundity and fertility). If genotypes differ in their viability then the relative frequency of genotypes will differ in the next generation. For this reason, one simple measure of fitness is considered to be the number of offspring produced by a specific genotype relative to another (FUTUYMA 1998).

Various natural populations of insects are made up of individuals of different phenotypes, more or less similar, whose relative frequency

remains fairly constant. The relative frequency of phenotypes is genetically determined as the result of mating between individuals of the same populations. The phenotypes will persist as long as different genotypes are favorably selected in different parts of the ecosystem or at different times (BUSKIRK et al. 1997; RICKLEFS 1990). This phenomenon is called genetic polymorphism (MAJERUS 1994; FUTUYMA 1998). The genetic polymorphism determines the existence of geographical races, subspecies, seasonal variation in the relative frequency of phenotypes, and the occurrence of rare phenotypes, which otherwise would be eliminated from populations (MAJERUS 1994).

Harmonia axyridis Pallas, like other ladybird beetle species, shows a highly polymorphic elytral pattern. Some of its phenotypes were previously considered different species and/or different genera (KOMAI 1956). The phenotypes

are classified in two main groups; (i) the *succinea* group, or light forms, characterized by the presence of elytras with color varying between shiny red and brown, and by the presence of a variable number of spots between 0 and 19, and (ii) the dark or melanic group, characterized by the presence of red spots on a dark background. The melanic group includes the phenotypes *conspicua*, *spectabilis* and *aulica* (TAN 1946, 1949; KOMAI 1956).

Different populations of *H. axyridis* show considerable geographic variation in the relative frequency of phenotypes (so called micro-geographic variation). In China and Manchuria, the phenotypes *succinea*, *conspicua* and *spectabilis* are very frequent (TAN 1946, 1949; KOMAI 1956). In Japan, *conspicua* and *spectabilis* phenotypes are the most frequent while in Korea the *succinea* phenotype predominates (DOBZHANSKY 1933; KOMAI 1956; OSAWA & NISHIDA 1992). On the other hand the phenotype *aulica* is at low frequency or is even absent in certain regions (DOBZHANSKY 1933). Very rare phenotypes such as *corvine* may also occur in natural populations (MADER 1932 in TAN 1946).

Some attempts have been made to relate elytral phenotypes in *H. axyridis* with geographical and seasonal factors (KRYLTSOV 1956; ABBAS & NAKAMURA 1985; ABBAS et al. 1988; OSAWA & NISHIDA 1992; HODEK & HONÉK 1996). However, the precise selective forces associated with the phenomenon are still very difficult to determine (MUGGLETON 1978). Macro-geographical variation of Asian populations of *H. axyridis* is apparently related to climatic factors, whereas micro-geographical variation, that is, differences in the relative frequency of elytral phenotypes between host plants in the same habitat, appear mostly related to food availability (KOMAI & HOSINO 1951; KOMAI 1956). Seasonal variation in the proportion of dark and light forms in Japanese populations has also been observed (TAN 1949; OSAWA & NISHIDA 1992). OSAWA & NISHIDA (1992) demonstrated a significant increase in the relative frequency of light phenotypes during spring and early summer. This result, supported by laboratory experiments, suggest the occurrence of assortative mating between different phenotypes. KOMAI (1956) reported long-term

changes in the relative frequency of elytral phenotypes in a population at Suwa, Japan. This author suggested that these change was due to natural selection, probably in relation to the milder winters in Suwa in more recent years.

Those observations suggest that polymorphism in *H. axyridis* can be adaptive and that the relative frequency of phenotypes in different populations could be related to their differing fitnesses in different environments. Recently, we showed that the *nigra* and *aulica* phenotypes of *H. axyridis* differ in their fitness (SOARES et al. 2001). Nevertheless remained to be tested the possibility that the most common phenotypes have highest fitnesses. We hypothesized that (i) a particular genotype that confers specific coloration pattern could also confer other specific attributes, as stated by LAMANA & MILLER (1995) and (ii) the most abundant phenotypes in Asian populations should show a higher fitness than the less abundant and rare phenotypes. We therefore predicted that the rare phenotype *nigra* [(h^2)], should show a lower performance than the less frequent, *aulica*-1 ((h^{A1})), or the most abundant *succinea* (h), *succinea*-3 ((h^3)) and *conspicua*-1 ((h^{C1})).

MATERIAL AND METHODS

Biological material

The phenotypes of *H. axyridis* individuals came from mass rearing at 25 ± 1 °C, 75 ± 5 % RH and photoperiod of 16L:8D. Coccinellids were fed on a diet of *Aphis fabae* Scopoli and eggs of *Ephestia kuehniella* Zeller. Field collected individuals of *H. axyridis* were added regularly to minimize inbreeding.

We used two phenotypes classified in the *succinea* group, *succinea* (h) or s0 and *succinea*-3 ((h^3)) or s9 and three classified in the melanic group, *conspicua*-1 ((h^{C1})) or c1, *aulica*-1 ((h^{A1})) or au and *nigra* [(h^2)] or ni. These forms occurred naturally in our mass rearing.

Biology of adults: longevity, body-weight and reproductive parameters

In order to evaluate the biology of adults of different phenotypes we sexed and paired 200 individuals of five phenotypes, thereby creating

20 couples for each phenotype. Each couple was isolated in a 60 ml Petri dish Mino-Gaillard (Ø: 5 cm, height: 3 cm). A daily surplus of apterous *A. fabae* females was provided. Egg clusters were removed from Petri dishes every day. All individuals were monitored until they death. The experiment was performed at 25 ± 1 °C, 75 ± 5 % RH and a photoperiod of 16L:8D, under fluorescent lamps (Sanyo FL 40 SS W/37). Different biological features were evaluated: longevity, percentage of fertile couples, number of egg clusters, number of days of oviposition, total and daily fecundity, daily fertility (including sibling cannibalism), percentage of hatching, sterile and embrionate eggs (3 days after the last hatched eggs), sex ratio and body-weight. Body-weight was determined for the parental generation (P), in the first day after emergence and in the first day of sexual maturation, and also for a pool including both parental and progeny of the same elytra, in the first day after emergence. Adults were weighed to an accuracy of 10^{-4} mg on a Mettler AM 50 analytical balance. Longevity, fecundity, fertility (including sibling cannibalism) and percentage of hatching were compared. Proportions were $\arcsin\sqrt{\%}$ transformed (ZAR 1984). One-factor ANOVA was used to compare longevity, fecundity, fertility (including sibling cannibalism), percentage of hatching and body weight. All statistical tests were done using JMP (SALL et al. 2001). The χ^2 test was used to compare the sex ratio in each phenotype.

Raw multivariate data were arranged in a square matrix, assigning individuals to rows and biological features (variables) to columns, and standardised (ROHLF 1994; FIELD et al. 1982). Individuals were compared using euclidean distances arranged in a symmetrical matrix of association (ROHLF 1994; LEGENDRE & LEGENDRE 1984; CLIFFORD et al. 1975), followed by a cluster analysis, using Unweighted Pair-Group Arithmetic Average (UPGMA). Principal component analysis (PCA) was used, in order to reduce the dimensionality of the data, to a few artificial variables (principal components), linearly uncorrelated and arranged in order of importance, in terms of the variance accounted for. The final plot of both phenotypes and biological parameters permitted us to visualize

the contribution of the latter to the statistical separation of phenotypes based on their biological characteristics.

RESULTS

Biological attributes of the elytral phenotypes, including longevity and reproductive capacity of adults, are summarized in Table 1. The longevity of *nigra* females and males was significantly less than the other phenotypes. Longevity did not differ between *aulica* and *succinea* (s0 e s9) males, which lived longer on average than *conspicua* males. No significant differences were found among longevities of *conspicua*, *succinea* (s0) and *aulica* females. The lowest percentage of fertile couples was observed in the *nigra* phenotype. The *nigra* and *succinea* (s9) couples showed a significant lower fecundity than the other phenotypes (Table 1). The *nigra* couples showed the lowest value for daily fertility (including sibling cannibalism). That is, females of this phenotype produced fewest larvae per day, followed by females of *aulica* phenotypes. No significant differences in daily fertility were observed between *succinea* s0 and *succinea* s9. The highest fertility values were observed in *conspicua* couples (Table 1). *Nigra* and *aulica* females showed the lowest hatching percentage of eggs. The highest hatching percentage was observed in *conspicua* couples. *Aulica*, *nigra* and *succinea* s9 showed no significant differences in terms of percentage of embrionate eggs, nor were there differences between *conspicua* and *succinea* s0. We observed the highest percentage of sterile eggs for the *nigra* couples, followed by the *aulica* and *succinea* s9 couples. No significant differences were observed between *succinea* s0 and *conspicua* (Table 1). The proportion of females obtained from *conspicua* ($\chi^2=0.093$, $df=1$, $p=0.76$) and *aulica* ($\chi^2=39.68$, $df=1$, $p<0.0001$) couples was significantly higher a 50:50 sex ratio. However, no significantly differences from a 50:50 sex ratio were obtained in *nigra* ($\chi^2=0.093$, $df=1$, $p=0.76$), *succinea* (s0) ($\chi^2=0.477$, $df=1$, $p<0.489$) and *succinea* s9 ($\chi^2=0.253$, $df=1$, $p<0.614$) (Table 1).

Table 1

Comparisons of longevity, body-weight and reproductive attributes of five phenotypes of *H. axyridis* [conspicua (c1), aulica (au), nigra (ni) and succinea (s9 and s0)]. Means and standard errors followed by different letters indicate post-hoc significantly differences. Last column indicate F values, degrees of freedom and p value of ANOVA.

Biological parameters	Phenotypes					F=	df=	P=
	<i>conspicua</i> (c1)	<i>aulica</i> (au)	<i>nigra</i> (ni)	<i>succinea</i> (s9)	<i>succinea</i> (s0)			
Longevity (days)								
Males (LM)	66.6±6.3c	76.3±5.4b	43.6±3.4a	75.3±7.4b	79.7±6.8b	F=5.89,	df=4, 94,	P=0.0003
Females (LF)	62.2±6.5b	60.3±4.2b	30.7±3.1a	52.2±5.5c	64.8±4.5b	F=8.04,	df=4, 94,	P<0.0001
Percentage of fertile couples (FrC)	95.0	85.0	55.0	85.0	90.0			
Number of batches (Bt)	33.5±4.1b	31.1±2.7b	11.4±1.4a	24.0±2.4c	34.9±3.4b	F=10.8,	df=4, 94,	P<0.0001
Number of day of oviposition (Ov)	26.8±3.1b	25.4±2.0b	10.7±1.3a	18.7±1.8c	27.7±2.6b	F=10.3,	df=4, 94,	P<0.0001
Total fecundity (TFc)	885.3±96.6b	808.3±85.1b	311.8±47.7a	579.5±72.9c	815.8±90.8b	F=8.58,	df=4, 94,	P<0.0001
Daily fecundity (DFc)	34.4±2.0a	31.1±1.8a	27.3±2.1a	31.0±2.3a	28.0±1.8a	F=2.06,	df=4, 94,	P=0.0925
Daily fertility (DFr)	12.8±2.1d	4.0±1.0b	0.56±0.3a	7.1±2.0c	8.1±1.3c	F=9.35,	df=4, 94,	P<0.0001
Percentage of hatching (Ha)	47.2±2.7c	22.8±2.7b	2.3±0.5a	26.3±2.9b	39.4±2.7c	F=72.7,	df=4, 74,	P<0.0001
Percentage of embryos (Em)	7.6±0.7b	5.8±0.7a	5.6±0.7a	5.6±0.9a	7.4±0.7b	F=6.02,	df=4, 74,	P<0.0001
Percentage of sterile (St)	45.1±2.9a	71.2±3.4b	94.1±0.9c	68.2±3.3b	53.2±9.9a	F=39.9,	df=4, 74,	P<0.0001
Sex ratio (females/males) (Sr)	1.11	1.62	1.09	0.96	1.03			
Body-weight after emergence (mg)								
Males (W1M)	25.8±0.7a	27.4±0.6a	26.2±0.6a	25.2±0.6a	26.3±0.3a	F=2.07,	df=4, 99,	P=0.091
Females (W1F)	32.1±1.2a	32.3±0.6a	31.3±0.6a	34.5±0.7a	32.2±0.4a	F=2.77,	df=4, 99,	P=0.310
Body-weight in the first day of sexual maturation								
Males (W2M)	31.7±0.8a	33.7±0.7a	31.8±0.6a	31.3±0.8a	32.3±0.3a	F=1.97,	df=4, 99,	P=0.105
Females (W2F)	44.3±1.6a	44.2±1.4a	44.6±0.9a	43.5±1.1a	43.9±0.6a	F=0.21,	df=4, 99,	P=0.935
Body-weight after emergence (P+F)								
Males (W3M)	26.2±0.4a	33.2±0.4b	25.8±0.4a	26.9±0.3a	26.6±0.3a	F=62.65,	df=4, 458,	P<0.0001
Females (W3F)	32.8±0.5a	38.2±0.5b	31.5±0.6a	32.3±0.4a	33.7±0.4a	F=31.11,	df=4, 468,	P<0.0001

Among the three estimates of body weight, significant differences are evident between males and females. Only the pooled estimate, included weights of both parents and progeny of the same elytral genotype, showed a significant difference among phenotypes: both females and males were heavier for *aulica* than for other phenotypes.

The UPGMA dendrogram using of euclidean distances, based on standardized biological data, cluster C1, S0 and S9, apart from both *aulica* and *nigra* phenotypes (Fig. 1).

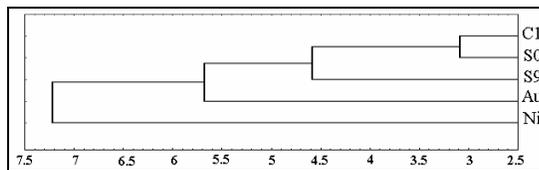


Fig. 1. UPGMA cluster of elytral phenotypes, based on euclidean distances using standardized biological parameters. Symbols are those presented in the text (Material and methods).

This grouping is also supported by the results of principal component analysis (PCA) that reduced the multidimensional data set to two principal axes, which explained 81.7% of the variance. The main contributions for the first axis

correspond to parameters related to longevity, fecundity and fertility, which could be associated to a general fitness dimension. On the other hand, the main contributions to axis 2 correspond to sex ratio of the offspring and body weight, with special importance of both sexes for W3, and males W1 and W2. While the first axis clearly separates the phenotype *nigra* from the other phenotypes, axis 2, discriminates phenotype *aulica*, and to lesser extent, phenotype s9, from the core group made by c1 and s0 (Fig. 2).

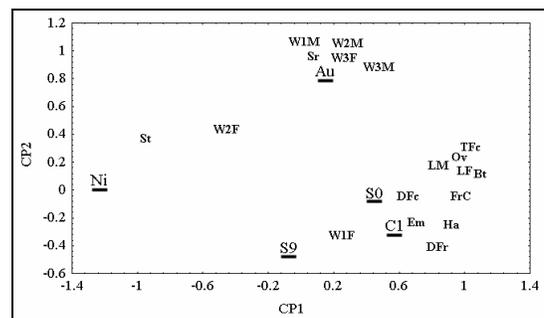


Fig. 2. PCA ordination was based on biological data of *H. axyridis*. PC1 and PC2 refer to the first principal components that explain, respectively, 52.5 % and 29.2 % of the variance. Elytral morphotypes are underlined. Symbols correspond with those in Table 1.

DISCUSSION

Stability of genetic polymorphism in a population is a consequence of the balance of diverse selective forces (SHEPPARD 1975). Most of the published studies focus on one selective factor thought to be responsible for the success of a particular phenotype relative to another. Direct or indirect fitness evaluation of phenotypes, under diverse ambient conditions, has rarely been done.

Biological control programs generally focus on the selection of the best parasitoid or predator species to be introduced based on criteria related to their capacity to have substantial impact on a pest population (WAAGE & MILLS 1992). However, selection of the most fit phenotypes of a selected species is rarely attempted (SOARES et al. 2001). Selection of the best phenotype will depend on the biological control strategy used. Certain biological parameters, such as reproductive capacity, will favor population establishment and predator-prey stabilization and may be most desirable in classical biological control programs, whereas short-term efficacy may be favored in inundative biological control programs. Our study showed that, under our experimental conditions, phenotypes of *H. axyridis* apparently differed in fitness. The most abundant phenotypes in nature, *succinea* and *conspicua*, were also the phenotypes with higher longevity, fertility, fecundity and percentage of hatching, in a clear contrast with the rare/less abundant phenotypes of *nigra* and *aulica*, which confirm our hypotheses that the most abundant phenotypes in Asian populations should show a higher fitness than the less abundant and rare phenotypes. Our results suggest, also, that *conspicua* and *succinea* (s0 and s9) individuals should be used in classical biological control programs because they have a greater longevity and higher reproductive capacity. These characteristics favor population adaptation and stabilization.

Temperature determines longevity in ectothermic species. In coccinellids longevity generally decreases with the increase of temperature. This effect was reported for *C. novemnotata* Herbst (MCMULLEN 1967), *Coleomegilla maculata lengi* (WRIGHT & LAING

1978) and *Olla v-nigrum* (Mulsant) (KREITER & IPERTI 1984). Despite the fact that experimental temperature was fixed at 25 °C, *nigra* phenotype was found to have a relatively short adult lifespan. This could be a disadvantage in biological control programs, from a practical point of view, since the decrease in time spent in foraging activity and ovipositing may reduce its fitness.

For polymorphic species, such as *H. axyridis*, the degree of melanization can also be an important factor affecting longevity. Melanic forms can reach a higher body temperature than non-melanic forms (DE JONG et al. 1996), and consequently they can reach higher metabolic rate and biological activity (DIGBY 1955; LUSIS 1961; DIXON 1972; BENHAM et al. 1974; MUGGLETON et al. 1975; WILLMER & UNWIN 1981; BRAKEFIELD & WILLMER 1985; STEWART & DIXON 1989; DE JONG et al. 1996). Our experiment was performed using low radiation fluorescent lamps. We therefore doubt that melanism decreased longevity of *nigra* through elevated body temperature. Instead, we suggest that longevity may be directly determined genetically, and the short lifespan of *nigra* may be adaptive when the environment is characterized by low temperature.

Indeed macro-geographic variation in Asiatic populations of *H. axyridis* is associated with climatic factors, such as temperature, as the result of physiological adaptations. In cold regions the dark phenotypes are, generally, more frequent than the light forms (KOMAI 1956).

Every species of coccinellid has genetically fixed rates of fecundity and egg sizes that are also affected by food availability and environmental conditions. If food supply is limited, coccinellids decrease their fecundity but will maintain egg size (HODEK & HONĚK 1996). More voracious and heavier coccinellid females have more resources to invest in egg production (FERRAN et al. 1984; HODEK 1993; STEWART et al. 1991a, b; SOARES et al. 2001). Observed differences in body weight of *aulica* adults versus adults of other phenotypes may reflect the particular dietary condition of our experiment. It remains to be investigated whether the same differences will occur in mass rearing programs. Nevertheless, given (1) the absence of significant differences in

female body weight among the parental generations of the different phenotypes and (2) the significant differences between fecundity and fertility, among the various phenotypes, our results suggest that reproductive capacity of phenotypes could be correlated with environmental conditions and prey quality. Thus additional experiments using other temperatures and prey should be performed.

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