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Phenotypic plasticity of elytron length in wingless two-spot ladybird beetles, *Adalia bipunctata* (Coleoptera: Coccinellidae)

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Abstract. Winglessness in the two-spot ladybird beetle *Adalia bipunctata* (L.) is determined by a single locus with the wingless allele recessive to the winged wildtype allele. The expression of the wingless trait is highly variable, with individuals missing a variable part of elytra and flight wings; the elytra and wings appear to be truncated rather than miniature in form. The degree of winglessness is partly determined genetically. Here we report on the phenotypic plasticity of the degree of winglessness. The environmental effect on elytron length relative to maximal elytron length in wingless phenotypes was studied by rearing offspring of single pair crosses of this form at a low (19°C) or high (29°C) temperature. Offspring reared at 19°C showed relatively longer elytra than those reared at 29°C.

INTRODUCTION

Winglessness has evolved many times in insects in response to a change in ecological conditions, for example on high mountains or in caves (Roff, 1990). Although the evolutionary forces and life-history trade-offs that promote winglessness have been addressed by several researchers (e.g. review in Harrison, 1980; Roff, 1984), the developmental mechanisms of winglessness are not well understood.

Wingless morphs of *Adalia bipunctata* are rarely found in the wild. In contrast to many other insects whose reduced wings resemble miniature normal wings (Darlington, 1936), both reduced elytra and flight wings of wingless *A. bipunctata* seem to be truncated (unpubl. data). Marples et al. (1993) showed for an individual found near Utrecht in The Netherlands that the presence of the wingless character is controlled by a recessive allele at a single locus.

The wingless trait shows variable levels of phenotypic expression, resulting in a variable degree of winglessness (DWL), with wingless individuals missing all or a variable part of elytra and flight wings. For a new wingless stock established from the same population, Ueno et al. (2004) found a significant family effect on the phenotypic expression of winglessness, indicating that the degree of winglessness is partly dependent on the genetic background. Thus the presence of the trait is controlled by one gene but the expression of the trait is probably controlled by more genes (i.e modifier loci), resulting in a variable degree of winglessness.

In many other insect species it has been shown that wing development is also strongly influenced by environmental factors. For example, relative wing length can vary with temperature (Stanley, 1935; Hosoi, 1954; Van den Heuvel, 1963; Nakao, 1993; Sakashita et al., 1995; Noach et al., 1996) and the incidence of macroptery (fully developed wings, as opposed to brachyptery: reduced wings) in dimorphic or polymorphic insects is strongly dependent on environmental factors, such as temperature, day-length and crowding (overviews in Honěk, 1976, 1981; Harrison, 1980; Aukema, 1986; Nakao, 1994; references in Olvido et al., 2003). The present study was performed to investigate the effect of an environmental factor, rearing temperature, on the degree of winglessness in *A. bipunctata*.

MATERIAL AND METHODS

One wingless *Adalia bipunctata* (L.) male, found at de Uithof, Utrecht, The Netherlands in 2000, was used together with wildtype ladybird beetles from the same locality to generate a laboratory stock of wingless and heterozygote ladybirds. This stock was outcrossed in at least three generations with different groups of wildtype individuals from the same locality to minimise inbreeding. The final group of wildtypes used for outcrossing included a single heterozygote for the wingless character. All the wingless alleles in the wingless stock were thus founded from a total of three wingless alleles collected in the wild.

To establish the material used in the present experiment families were raised from four pairs of males from this stock heterozygote for the wingless character that were each crossed with a wingless female. The pairs were kept in a climate cabinet at a constant temperature of 20.5°C and 16L : 8D. Eggs were collected three times a week during 19 consecutive days in 2004. Eggs collected during the first nine days were stored at 10°C and then all moved back to the climate cabinet at day 12, to decrease the variation in developmental stage among larvae. All other eggs (days 10–19) remained at 20.5°C. Six days after hatching, larvae were randomly divided over temperature regimes of 19°C (RH ± 60%) or 29°C (RH ± 63%). They were then reared individually through to adults in 55 mm petridishes. They were fed *Ephestia kuehniella* Zeller eggs (kept frozen until required) three times a week and moved to new dishes once a week to maintain hygiene. Newly-emerged adults were returned to 20.5°C and fed with *E. kuehniella* eggs for about a week before they were frozen at –80°C.

All wingless offspring were sexed and the expression of the wingless trait was estimated using a categorical system for the degree of winglessness (DWL) that differed from that of Ueno et al. (2004). Because the outer (officially referred to as "anterior") and medial ("posterior") part of an elytron are often unequally developed, and the left and the right elytron are not

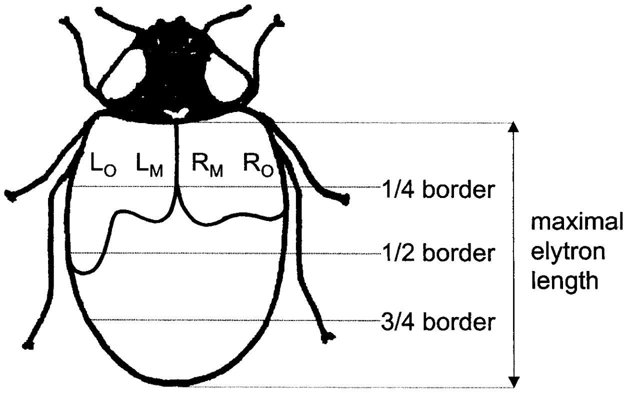

Fig. 1 Schematic drawing of a wingless ladybird showing the borders of ¼, ½ and ¾ of the maximal elytron length, as used to determine the length of medial and outer parts of both elytra (LO = left outer, LM = left medial, RM = right medial, RO = right outer).

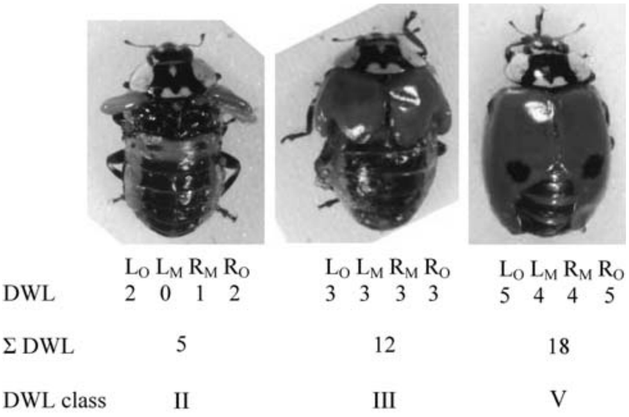

Fig. 2 Wingless *Adalia bipunctata* and the values for the degree of winglessness (DWL) as a measure for expression of the wingless trait. DWL values refer to the length of medial and outer parts of each elytron relative to the maximum elytron length (LO = left outer, LM = left medial, RM = right medial, RO = right outer; 0 = no elytron visible, 1 = only a round bud visible, 2 = flat elytron up to ¼ of maximum, 3 = elytron > ¼ up to ½ of maximum, 4 = elytron > ½ to ¾ of maximum, 5 = > ¾ of maximum to complete elytron). The Σ DWL determines the DWL class for the individual ladybird.

always similar, we took the development of the outer and the medial part of each elytron into account separately. We determined the DWL of the reduced part of each elytron by estimating its length relative to the maximal elytron length, using the distance between the caudal bound of the pronotum and the end of the abdomen along the longitudinal axis of the body as a measure for a complete elytron (Fig. 1). When the elytron was pointing outwards, it was, in a virtual way (i.e. in the observer's mind), folded back over the abdomen in order to estimate its length. We scored the DWL by eye using a binocular microscope (0 = no elytron visible, 1 = only a round bud visible, 2 = flat elytron up to ¼ of maximum, 3 = elytron > ¼ up to ½ of maximum, 4 = elytron > ½ to ¾ of maximum, 5 = > ¾ of maximum to complete elytron, Figs 1–2). The sum of the four DWL scores (Σ DWL) finally determined the DWL class of an individual ladybird (class 0 if Σ DWL = 0, class I if Σ DWL = 1–4, class II if Σ DWL = 5–8, class III if Σ DWL = 9–12, class IV if Σ DWL = 13–16 and class V if Σ DWL = 17–20). This method was initially tested by two observers who independently scored the DWL of 107 *A. bipunctata* ladybirds twice. DWL was highly repeatable both within and between the two observers (Σ DWL observer A: $r_s = 0.925$, $p < 0.001$; Σ DWL observer B: $r_s = 0.970$, $p < 0.001$; Σ DWL between observer A and B: $r_s = 0.970$, $p < 0.001$).

Because ladybird numbers per family were small, for statistical analysis we pooled the sexes (in all data combined males do not differ from females, see results). The effect of the cooling of some eggs (days 1–9) on the DWL class was first investigated by a heterogeneity chi-square test, separately for eggs later reared at 19°C and those reared at 29°C. Subsequently, the variation in DWL classes across families was analysed by heterogeneity chi-square tests, pooling DWL classes with expected numbers below one. We finally tested the variation in DWL classes across temperatures per family.

RESULTS

There was no significant difference in the frequency classes for DWL between the sexes in each temperature treatment (19°C: $\chi^2_4 = 9.384$, $p > 0.05$; 29°C: $\chi^2_2 = 0.689$, $p > 0.05$). There was also no effect of the cooling period that some eggs experienced on DWL class (19°C: $\chi^2_4 = 4.232$, $p > 0.05$; 29°C: $\chi^2_2 = 5.583$, $p > 0.05$). Therefore, we did not analyse sex or cooling period in the following analysis.

Table 1 shows the frequency distribution of DWL classes for each family at each temperature. The families are homogeneous at 19°C ($\chi^2_6 = 6.944$, $p > 0.05$) but not at 29°C ($\chi^2_6 = 14.862$, $p < 0.05$). Nevertheless, families each show a similar trend across temperatures, with individuals reared at 19°C having longer elytra than those reared at 29°C, and two families show highly significant heterogeneity chi-square values (Table 1). The

TABLE 1. Degree of winglessness (DWL) of *Adalia bipunctata* offspring from four families at two rearing temperatures. Differences in DWL class across temperatures were tested by heterogeneity chi-square tests. T = temperature, N = total offspring number, number in DWL class = offspring number per DWL class, hetχ^2 = heterogeneity chi-square value, d.f. = degrees of freedom.

Family	T (°C)	N	number in DWL class						het χ^2	d.f.	p
			0	I	II	III	IV	V			
1	19	22	1	11	5	4	1	0	2.849	3	N.S.
	29	14	2	9	2	0	0	1			
2	19	13	0	5	3	3	2	0	3.968	2	N.S.
	29	14	0	7	6	1	0	0			
3	19	11	1	3	4	3	0	0	4.773	1	<0.05
	29	4	1	3	0	0	0	0			
4	19	21	1	3	10	4	3	0	22.153	4	<0.001
	29	14	5	9	0	0	0	0			

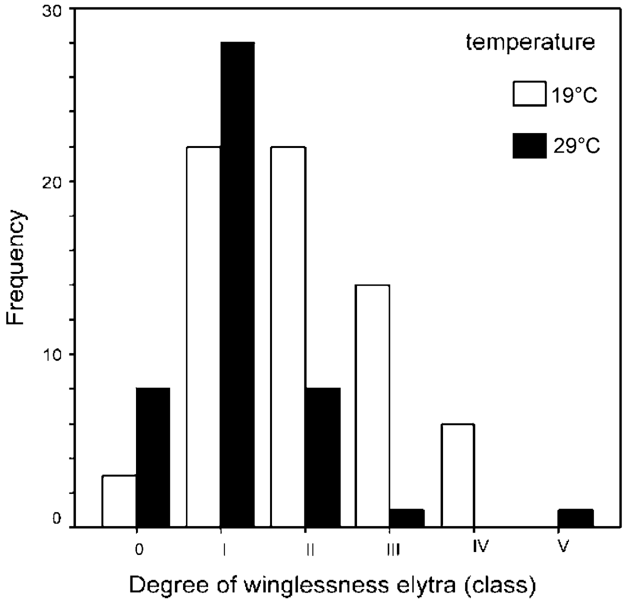

Fig. 3 Frequency distribution of degree of winglessness (in classes) of elytra from wingless *Adalia bipunctata*. Bars represent numbers of wingless offspring from the pooled data for four families that were randomly divided over two temperature regimes during larval and pupal development (19°C or 29°C).

overall trend towards longer elytra at lower temperature is illustrated in Fig. 3.

DISCUSSION

The effect of temperature on elytron and wing length

Our results show an effect of temperature on the relative elytron size in wingless *A. bipunctata*. The increase in relative wing length as a result of rearing nymphal stages at lower temperatures as we observed has also been found in *Drosophila* (Stanley, 1935; Noach et al., 1996; Morin et al., 1999) and in mosquitoes (Hosoi, 1954; Van den Heuvel, 1963). It is thought that the switching between morphs in wing dimorphic insects can be influenced by temperature, too. Thus, there are some examples of wing dimorphic species that show an increased incidence of macropterous morphs under cold conditions, in agreement with the above trend (references in Southwood, 1961). However, the incidence of macropterous morphs usually decreases with lower temperatures. Examples include: (1) during rearing at lower temperatures in heteropteran species (Honěk, 1981; Sasaki et al., 2002) and thrips (Nakao, 1994); (2) with lower seasonal and climatic temperatures in a cricket (Roff & Simons, 1997; references in Olvido et al., 2003) and a heteropteran species (Honěk, 1981); and (3) at higher altitudes in several heteropteran species (references in Southwood, 1961). Moreover, a decrease in relative wing length with declining rearing temperature has been reported for species of thrips (Nakao, 1993; Sakashita et al., 1995) and for vestigial mutants of *Drosophila* (Stanley, 1935).

Mechanisms of wing development

These contrasting examples indicate that the mechanisms of wing development may differ substantially among insects, and that environmental factors can act differently upon them, for example through juvenile hormone titres (Southwood, 1961).

Some other Coleopteran species are known to have natural brachypterous morphs with variable truncated flight wings (but not elytra) similar to that of *A. bipunctata* (Darlington, 1936). However, to our knowledge there are no descriptions of wing and elytron development in these species. It will be difficult to explain the observed temperature effect on elytra length as long as the development of elytra and wings in wingless *A. bipunctata* remains undescribed. We are, therefore, currently researching their development in winged and wingless *A. bipunctata*.

The egg stage does not seem to be a critical stage in determining wing length since there was no effect of cooling eggs as long as ten days on final elytron length. The first six larval days, covering the first, and part of the second larval instar, are also apparently not crucial. This is in agreement with results of Van den Heuvel (1963) and Hosoi (1954) who respectively found that adult wing length of mosquitoes was least affected by temperature in the first larval instar, and most strongly from the fourth instar onward. In addition, Honěk (1981) showed that wing development in a wing dimorphic heteropteran species was strongly affected by temperature in fourth and fifth instar larvae, whereas it was hardly affected by treatment in first to third instar larvae.

Phenotypic plasticity of elytron length and canalisation

The observed phenotypic plasticity in wingless *A. bipunctata* is not seen in winged individuals (all winged offspring reared at 19°C and 29°C have completely developed elytra). This is consistent with observations that mutant stocks have often more variable phenotypes than does the wildtype (e.g. Scharloo, 1991; Stearns & Kawecki, 1994; Hermisson & Wagner, 2004). This might be because development is not as effectively canalised, making the mutant stock more sensitive to genetic and environmental disturbances (Waddington, 1959; but see Hermisson & Wagner, 2004).

Bégin et al. (2004) showed that micropterous crickets display more phenotypic variation in wing morphology than macropterous individuals over a range of rearing temperatures. They explain this by a less canalised development and maintenance in the micropters. The micropterous crickets also have a higher fecundity than the macropterous ones. Therefore, Bégin et al. (2004) suggest that the micropters have, among other costs, a less canalised development and maintenance as a trade-off for a higher fecundity. Whether this explanation might also hold for *A. bipunctata* requires investigation by examining the patterns of such trade-offs. So far studies of wingless *A. bipunctata* have revealed no reproductive advantage other than a shorter preoviposition period compared to wildtype individuals (Ueno et al., 2004).

Environmental and genetic components affect elytron length

Previous research indicated that the relative elytron length in wingless *A. bipunctata*, as a measure for the degree of winglessness, is partly influenced by a genetic component (Ueno et al., 2004). The heterogeneity in elytron length we found across wingless families at 29°C supports these findings. However, our results suggest that there is also an environmental component that determines relative elytron length in wingless *A. bipunctata.* Larger families will be needed to confirm whether the pattern is consistent across the sexes. Although all four families showed a trend towards longer elytra at lower temperature (Fig. 3), it was only significant in two families (Table 1). These differences suggest a family-by-environment effect. A detailed description for a larger number of families would enable an examination of the variability of the plasticity. This research can contribute to the understanding of the mechanisms underlying wing development and to the evolution of development of winglessness in insects.

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