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The inheritance of a wingless character in the 2spot ladybird (*Adalia bipunctata*)

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Abstract

A viable wingless 2spot ladybird *Adalia bipunctata* (L.) was found in the wild. Breeding through four generations revealed that the wingless trait was controlled by a recessive allele which displays variable levels of expression. The wingless ladybird is discussed in relation to its potential as a biocontrol agent. One ladybird also occurred in this stock which is suggestive of a supergene controlling the colour polymorphism in this species.

Introduction

The 2spot ladybird *Adalia bipunctata* (L.) is a very abundant colour-polymorphic species in Europe. Most populations include three main colour morphs: *typica* which has two black spots on a red background; and two melanic forms, *quadrimaculata* with four, and *sexpustulata* with six red spots on a black background. These main colour forms, *typica* and melanic, are controlled by a single gene (Lus, 1932; Majerus & Kearns, 1989), and in most populations the melanic alleles are dominant to the *typica* (see reviews Komai, 1956; Brakefield, 1985).

Occasionally in these populations a 'wingless' form is found, which lacks all or part of the elytra and flight wings. Such individuals have been reported by Majerus and Kearns (1989) but were always weak, and could not turn over when they fell on their backs, so normally died (Majerus pers. comm.). Thus the genetic basis of this trait had not previously been investigated. This study reports its inheritance through several generations.

Forms with shortened wings occur in other genera of ladybirds (Hammond, 1985) and many other groups of insects show micropterous forms (review Roff, 1986). In most of these cases the genetic basis is a polygenic system, although there are a number of examples where the trait is controlled by a single gene. In these, the wingless allele is usually dominant (Roff, 1986).

An important use of the wingless form of the 2spot ladybird relates to its potential as a biocontrol agent against aphids. Ladybirds, especially as larvae, consume large numbers of aphids daily (Ellingsen, 1969; Hämäläinen *et al.*, 1975) and have therefore been proposed as a control agent for crop infestations of aphids (Markkula *et al.*, 1972; Honěk, 1980, 1982). However, tests on their use in greenhouses have shown that although the ladybirds reduce the infestation on some crops, they quickly fly off the plants to the windows, so regular reintroductions are necessary (Hämäläinen, 1977). If a pure strain of wingless ladybirds were used in the initial treatment of the crop, the adults would not be able to fly away, providing the potential for longer term control.

We present in this paper evidence that the wingless trait is inherited, and some information about the gene's expression. This represents a considerable step towards its development for biocontrol purposes.

The wingless strain

A wingless individual 2spot ladybird was collected from a wild population in The Uithof, Utrecht, The Netherlands. It was found at ground level under lime trees *Tilia* sp., with a large population of normal melanic and typica 2spot ladybirds, in September 1990. The colour of its pronotum suggested that it was a melanic form (cf. Fig. 1). Its abdomen and pronotum appeared normal, but the elytra were completely absent, and the flight wings reduced to small buds. This did not seem to be due to damage as no signs of tearing or discoloration were visible when the individual was inspected under the microscope.

Unlike those reported by Majerus and Kearns (1989), this individual, and all its subsequent offspring, could turn over by sudden flicking of the abdomen, allowing it to grip the ground above its head and somersault back onto its feet. It could also turn over by curling itself sideways, and then rolling over. This latter behaviour is not known in normal winged forms of the 2spot, as they turn themselves over by opening their wings, and cannot turn sideways as their elytra prevent lateral bending.

Breeding

The individual was a male, but unusually large, being of a size more typical for females of this species (Majerus & Kearns, 1989). It mated readily with a normally winged female, a virgin *typica*, bred from a field population from Breda, The Netherlands.

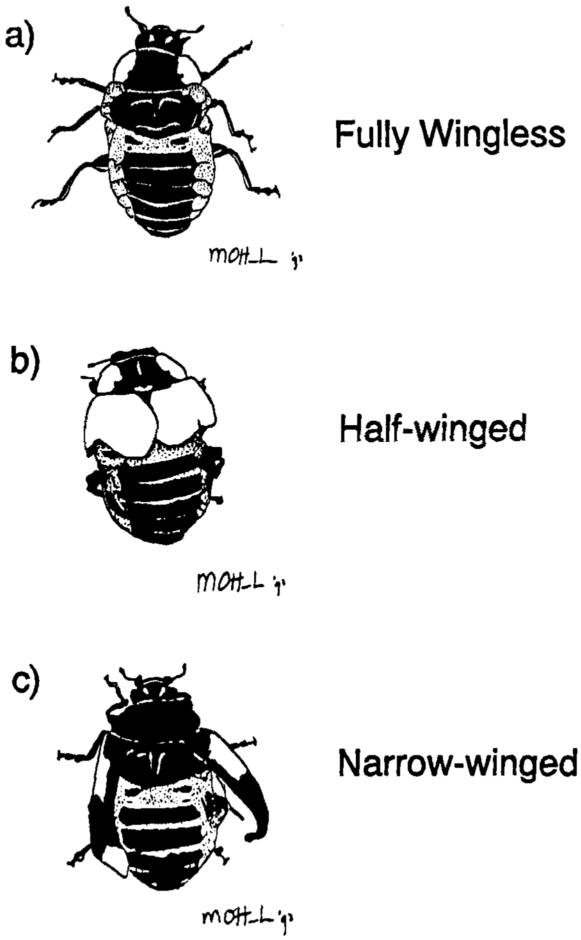

Fig. 1. The three main forms of the wingless ladybird: a) fully wingless (*typica*); b) half-winged (*typica*); c) narrow-winged (melanic). (Drawings from photographs of live beetles).

F1 generation

The 12 resulting F1 offspring were all fully winged, as would be expected if the wingless state were controlled by a recessive gene. This observation is, however, also consistent with the hypothesis that the wingless individual had simply been damaged. There were some F1 individuals with each of the colour morphs, 10 *typica* and 2 melanics. As the melanic allele is dominant over *typica* (Lus, 1932), the presence of *typica* offspring suggests that the melanic wingless ladybird was a heterozygote for the colour gene, although the number of melanics produced was unexpectedly low.

F2 generation

The F1 generation were paired randomly with each other to give an F2 generation. This generation comprised both melanic and *typica* forms of both wingless and normally winged ladybirds. The wingless forms varied in the extent and type of their winglessness, some having only reduced elytra, and some being as completely wingless as the original parent (see Table 1 and Fig. 1); representative specimens have been placed in the National Museum for Natural History in Leiden, The Netherlands. The proportions of normal to wingless ladybirds in this generation were 14 wingless to 34 normally winged, which is consistent with the 1:3 proportions expected for a recessive single allele ($\chi^2 = 0.44$, df = 1 NS). It appeared not to be sex or colour morph linked since both sexes and morphs occurred among the wingless and normal progeny. Some died before they were sexed or recorded for colour morph, but of the survivors, the ratios were: 5 *typica* to 4 melanics for the wingless, and 30 *typica* to 4 melanics for the winged; and 7 male to 2 female for the wingless and 22 male to 12 female for the winged.

The wingless individuals from the F2 generation which survived to breed are summarised in Table 1. The differing degree of winglessness in the individuals expressing the wingless gene is illustrated as three distinct types in Fig. 1, although some individuals were intermediate between these types. Such variable expression of a recessive trait is well known (see Strickberger, 1968).

Table 1. Surviving F2 wingless individuals with an indication of the degree to which they expressed the wingless trait

Beetle	Sex	Colour	Winglessness
1	M	*typica*	buds
2	M	*typica*	1/2 length
3	M	melanic	buds
4	F	melanic	narow
5	M	*typica*	buds
6	M	*typica*	narrow
7	M	melanic	buds
8	M	*typica*	1/2 length
9	F	melanic	buds

There also appeared in the F2 generation a winged ladybird which appeared to be intermediate between two of the colour morphs. Its pronotum was black, as found in the melanic forms, while its elytra were red with two black spots, as seen in the *typica* form. The elytra were also slightly darker than the normal *typica* elytra, and this became more marked with time, although there was no sign of the melanic elytral pattern appearing. The elytra remained red, but very dark. Unfortunately this individual died before it could be bred from. However, it provides an interesting indication that the 'single gene' for colour pattern may in fact be a supergene, occasionally being divided during recombination into parts coding for the elytral pattern and those encoding the pattern for the pronotum. The possible existence of a supergene is also relevant to the issue of whether this species is a polymorphic Batesian mimic (Brakefield, 1985). The darkened elytra has also been seen in a *typica* 2spot with an unusually dark pronotum, found in the wild. This phenomenon may indicate that the melanisation process is under separate control from that determining the colour pattern, since the darkening of the elytra continued despite the absence of melanic pattern.

F3 generation

The F2 wingless females were both mated with F2 wingless males in an attempt to breed directly for the wingless gene in the F3 generation. Unfortunately no viable offspring were produced by these pairs as there was a high failure rate in hatching of ladybird eggs in all stocks at that time.

Five of the F2 wingless males were mated with virgin winged females from a laboratory population which originated from Breda. This outcross was intended to strengthen the stock. Three of these pairs produced viable offspring, giving an F3 generation of 33 winged *typica* ladybirds. This result confirms the recessive nature of the wingless allele.

The winged offspring in the F2 generation were mated together to allow any carrying the wingless gene in heterozygote form to produce wingless individuals in the next generation. This stock was unfortunately lost due to food shortage.

F4 generation

The 33 winged *typica* ladybirds of the F3 generation, which should all have been heterozygous for the wingless gene, were placed into four breeding groups, with each of the three families represented in each group to minimise inbreeding. Each of these groups produced some viable young, although again hatching rates were low, and the numbers ranged from 2 to 26. The resulting F4 generation consisted of 42 winged individuals, and 3 wingless. The degree of winglessness again varied between individuals (see Table 2). The progeny from the four groups are shown in Table 2. Significantly fewer wingless ladybirds occurred in this generation than expected for a 1:3 segregation ratio ($\chi^2 = 8.07$, df = 1, $p < 0.01$). There are at least two possible explanations for this observation. It may reflect greater mortality among the wingless stock, during the egg, larval or pupal stages. However, if only the families in which any winglessness occurred are considered, then the ratios of adults produced do not differ significantly from 3:1 (G = 0.967, df = 1, NS) although the numbers are small. Thus it is possible that the wingless gene had somehow been lost from the other two families, but, where it occurs, it acts as a standard single recessive gene.

None of the F4 wingless ladybirds produced any viable offspring, and the wingless stock was terminated.

Table 2. Numbers of wingless and normally winged F4 individuals produced by each breeding group

	Winged	Wingless	Description
Group 1	5	1	narrow
Group 2	11	2	1/3 length 1/4 length
Group 3	2	0	
Group 4	26	0	
Total	42	3	

Conclusion

The breeding of this stock demonstrates that at least some of the occasionally occurring wingless adults found in the wild are homozygous for a recessive single gene which prevents normal development of the elytra and flight wings. This gene can be expressed by a number of deformities of the elytra, all of which would prevent efficient flight. As stocks breed true for this gene there is potential for the development of a strain of wingless ladybirds as an efficient biocontrol agent for greenhouse crop pests.

There is little evidence in the present study to suggest that this would not be straightforward since the failure to breed large numbers of the wingless stock in each generation was also reflected in our normal winged ladybirds at the time. However, as no offspring from wingless X wingless crosses were obtained, it is not known whether a pure strain can be produced.

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