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Genetic linkage between melanism and winglessness in the ladybird beetle *Adalia bipunctata*

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Abstract We report a case of genetic linkage between the two major loci underlying different wing traits in the two-spot ladybird beetle, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae): melanism and winglessness. The loci are estimated to be 38.8 cM apart on one of the nine autosomes. This linkage is likely to facilitate the unravelling of the genetics of these traits. These traits are of interest in the context of the evolution of intraspecific morphological diversity, and for the application of ladybird beetles in biological control programs.

Keywords *Adalia bipunctata* · Classical genetics · Linkage disequilibrium · Wing morphology

Introduction

Variation in insect wing morphology has long been studied from different perspectives. Examples include both fundamental studies in the context of the evolution and development of phenotypic variation, and applied studies in the context of pest control. The genetics underlying such variation can help to better understand the nature of the variation in either of the contexts. We report here genetic linkage of two morphological wing traits, melanism and winglessness, in the ladybird beetle, *Adalia bipunctata* (L.).

Melanism in ladybirds

Intraspecific variation in colour patterns of ladybird beetles (Coleoptera: Coccinellidae) has been studied for many decades (Majerus 1994, 1998; Dobzhansky 1924, 1933; Lusis 1961). Generally, the elytra (the pair of fore wings that are sclerotized in beetles) of ladybird beetles vary in the localization and extent of melanin, a dark pigment (Majerus 1998). In most species, there is a strong hereditary component determining melanism. There is temporal and geographic variation in frequencies of melanic individuals (Majerus and Zakharov 2000; Bengtson and Hagen 1977; Brakefield and de Jong 2011; Creed 1975; Michie et al. 2011; Timofeeff-Ressovsky 1940), and there is an ongoing debate about the nature of the selective forces maintaining colour polymorphisms in different species. Proposed theories include those related to thermal properties of black elytra ('thermal melanism') (Lusis 1961; Brakefield 1984b; Michie et al. 2010; Brakefield and Willmer 1985; De Jong et al. 1996), mimicry (Brakefield 1985), and mating preferences (Majerus et al. 1986). In the past two decades, the genetics underlying variation in melanism have been unravelled for several species of insects. Studies of *Drosophila* spp. have helped to decipher the melanin biosynthesis pathway, and the effector genes involved in this canonical cascade seem well conserved throughout the insects (reviewed by Wittkopp and Beldade 2009; True 2003; Van't Hof and Saccheri 2010). The mechanisms responsible for regulation of the positioning of

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melanin in space and time, are however, far more diverse (Wittkopp et al. 2003; Van't Hof et al. 2011), and have not been elucidated for ladybird beetles.

Winglessness in ladybirds

Some species of ladybird beetles also exhibit natural variation in wing length (Majerus 1994; Hammond 1985; Pope 1977). Little is known about the development and evolution of this trait (Lommen et al. 2009), but interest has grown in the potential use of wingless morphs in biological control programs involving ladybirds as natural enemies (Lommen et al. 2008; Obrycki et al. 2009).

Melanism and winglessness in *A. bipunctata*

In some natural populations of the ladybird, *A. bipunctata*, both elytral colour pattern and elytron length are polymorphic (Marples et al. 1993; Lusis 1961) (Fig. 1). With respect to the colour polymorphism, hundreds of different colour morphs have been described, but three predominate in nature: the typical, non-melanic morph which has red elytra with two black spots (*typica*), and the melanic morphs with black elytra and with either four (*quadrimaculata*) or six (*sexpustulata*) red spots (Brakefield 1984a; Majerus 1998). It has been proposed that variation in elytra colouring is determined by a series of alleles at a major supergene, hierarchical in dominance, with melanic morphs generally dominant over non-melanics (Majerus 1998). However, the location of this supergene in the genome, and the sequence of the alternative alleles at this locus, are still unknown.

Typically, elytra cover the entire abdomen and the folded flight wings of *A. bipunctata.* In contrast, in wingless morphs, both pairs of wings (elytra and flight wings) are truncated, such that the abdominal part of the abdomen is not entirely covered by the elytra (Lommen et al. 2009). Wingless morphs are rare, but have been recorded for at least one population in The Netherlands (Marples et al. 1993), and five in the United Kingdom (M. E. N. Majerus, personal communication). So far, no fitness advantages have been found for the wingless morphs. The wild type is favoured for several life history traits (Ueno et al. 2004), and wild-type females are more frequently mated by males than wingless ones (Lommen et al. in preparation). Therefore, the potential adaptive value of winglessness in *A. bipunctata* in nature, if any, is unknown. Nevertheless, wingless *A. bipunctata* have been shown to be successful in biological control of aphids (Lommen et al. 2008, 2012). Winglessness (the presence of the truncation) is determined by one major, single locus with the winged, wild type allele being dominant over the wingless one (Marples et al. 1993). The location of the locus and the sequence of its alternative alleles, remain obscure. In this paper, we examine the degree of genetic linkage between these two wing traits, elytral melanism and winglessness, in *A. bipunctata* in classical genetic crosses.

The study population

Our observations on linkage between the wingless trait and elytral colour were all made in a laboratory population of wingless *A. bipunctata*. The origin of this population is described in detail by Lommen et al. (2005). In summary, the ancestors of the population were all collected in the Utrecht region in The Netherlands, and one of them was wingless (homozygote recessive for the wingless trait). The frequency of the wingless alleles in the laboratory population was elevated to 100 % by selecting wingless phenotypes in the F2 generation and breeding them together. To maintain a diverse genetic background, they were outcrossed to wild-type beetles every now and then.

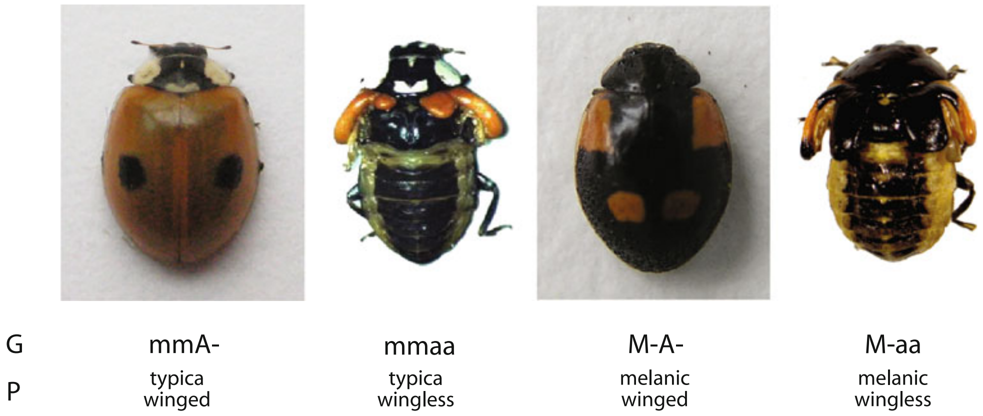

Fig. 1 Variation in elytral colour and winglessness in *A. bipunctata*. Below each photograph, the corresponding phenotypes (*P*) and genotypes (*G*) are given, with *letters* representing the alternative alleles at both loci (*M* melanic allele, *m* typical allele, *A* winged allele, *a* wingless allele), with *capital letters* dominant over *lower case letters*

Table 1 Linkage between the genes for elytral colour and winglessness in a laboratory population of *A. bipunctata*

cross	parental genotypes		F1 offspring numbers per genotype (observed (expected))						genetic linkage				
	♂	♀		total	mmaa^{a}	MmAa^{a}	mmAa^{b}	Mmaa^{b}	G	df	P	RF	map distance (cM)
group	MmAa mmaa	mmaa MmAa		2426	722(456)	1044(778)	365(631)	295(561)	499.96	1	0.000*	0.27	38.8
fam 7	mmaa	MmAa		376	122 (86)	138 (102)	61 (97)	55 (91)	56.32	1	0.000*	0.31	48.0
			♂	*191*	*57 (39)*	*75 (57)*	*31 (49)*	*28 (46)*	*27.74*	*1*	*0.000**		
			♀	*185*	*65 (47)*	*63 (45)*	*30 (48)*	*27 (45)*	*28.01*	*1*	*0.000**		
fam 9	mmaa	MmAa		358	112 (79)	132 (99)	46 (79)	68 (101)	49.27	1	0.000*	0.32	50.6
			♂	*167*	*51 (36)*	*62 (47)*	*20 (35)*	*34 (49)*	*22.23*	*1*	*0.000**		
			♀	*191*	*61 (43)*	*70 (52)*	*26 (44)*	*34 (52)*	*27.21*	*1*	*0.000**		
fam 16	mmaa	MmAa		172	58 (35)	75 (52)	21 (44)	18 (41)	53.23	1	0.000*	0.23	30.2
			♂	*100*	*42 (27)*	*38 (23)*	*9 (24)*	*11 (26)*	*38.55*	*1*	*0.000**		
			♀	*72*	*16 (9)*	*37 (30)*	*12 (19)*	*7 (14)*	*13.41*	*1*	*0.000**		
fam 18	mmaa	MmAa		36	12 (7)	15 (10)	7 (12)	2 (7)	10.79	1	0.001*		
fam 25	mmaa	MmAa		82	35 (25)	26 (16)	7 (17)	14 (24)	20.89	1	0.000*	0.26	35.9
fam 28	mmaa	MmAa		27	7 (6)	9 (8)	6 (7)	5 (6)	0.90	1	0.342		
fam 29	mmaa	MmAa		170	62 (39)	69 (46)	19 (42)	20 (43)	52.37	1	0.000*	0.23	30.7
			♂	*87*	*34 (22)*	*33 (21)*	*10 (22)*	*10 (22)*	*26.79*	*1*	*0.000**		
			♀	*83*	*28 (17)*	*36 (25)*	*9 (20)*	*10 (21)*	*25.25*	*1*	*0.000**		
fam 32	MmAa	mmaa		27	10 (6)	12 (8)	4 (8)	1 (5)	12.70	1	0.000*		
fam 46	MmAa	mmaa		45	17 (13)	13 (9)	6 (10)	9 (13)	5.12	1	0.024*		
fam 50	MmAa	mmaa		24	9 (6)	8 (5)	5 (8)	2 (5)	4.85	1	0.028*		
fam 58	MmAa	mmaa		50	12 (7)	25 (20)	7 (12)	6 (11)	9.87	1	0.002*	0.26	36.7

Data are shown in separate rows for one group cross and for each of eleven individual families (fam). For the four largest families, the results are additionally split for sons and daughters. For each cross, the first grey block gives the parental genotypes, followed by the distribution of genotypes in the F1 offspring in the white block, with letters corresponding to the alleles (*M* melanic allele, *m* typical allele, *A* winged allele, *a* wingless allele), and capital letters dominant over lower case letters. The second grey block gives details on the linkage. Recombination fractions and map distances are only given for families with at least 50 offspring. *G* value of G following a *G* test of independence, *df* degrees of freedom, *P* probability value resulting from the *G* test (significant *P* values are indicated by an asterisk); *RF* fraction of recombinant genotypes of the total offspring number; map distance according to Haldane's map function (in cM)

[a] Parental genotype

[b] Recombinant phenotype

Detection of linkage between melanism and winglessness

We initially observed a correlation between melanism and winglessness while breeding the wingless population. We had composed groups of virgin, melanic, winged individuals (heterozygote for both traits) of one sex, mixed with virgin, typical, wingless individuals of the opposite sex (homozygote recessive for both traits). The F1 generation of these group crosses constituted the two parental phenotypes (typical, wingless individuals, and melanic, winged individuals), and the two recombinant phenotypes (melanic, wingless individuals, and typical, winged individuals) (Fig. 1). In total, 2,426 offspring were scored for both traits. Since the wingless phenotype was less frequent than the winged one, expected frequencies of each of the four phenotypes were calculated based on allele frequencies in the total population of the F1 generation. 73 % of the F1 offspring corresponded to one of the parental phenotypes, which is a significant deviation from the expected frequency, and suggesting linkage disequilibrium (Table 1). From these numbers, map distance was calculated to be 38.8 cM following Haldane's map function (Haldane 1919).

Subsequently in one of the outcrossing events, the wingless laboratory population was enriched with a further copy of a wingless allele. It originated from an individual found at the same locality that proved to be heterozygous for the wingless trait, and so all three alleles used to establish our stock were likely to be copies of the same wingless allele. We then used the enriched stock to confirm genetic linkage in similar crosses, but instead using twenty individual families. Eleven of them were bred from a melanic, winged male with a typical, wingless female, and nine from a typical, wingless male with a melanic, winged female. All parents were virgin at the start of the experiment. Female ladybirds store sperm after mating, and lay eggs nearly daily throughout life (De Jong et al. 1998). F1 offspring were collected as long as the female laid fertilized eggs, bred to adulthood, and scored for elytral colour and winglessness. Offspring numbers varied widely from 4 to 376 with a median of 50 offspring. Because wingless *A. bipunctata* have lower longevity than winged ones (Ueno et al. 2004), the number of offspring of families with wingless mothers was lower than of those with a wingless father (Mann–Whitney U test, $U = 19.5$, $P < 0.05$). Otherwise, there were no differences between these two types of families. Overall, nine families yielded too few offspring to allow statistical analysis and were, therefore, excluded. The results from the other eleven families are summarized in Table 1 showing offspring numbers per phenotype, the significance of genetic linkage calculated by G tests of independences, and the estimated map distances. Ten of the eleven families showed significant linkage. A similar non-significant trend was seen in the other family. Four of the families were large enough to examine male and female offspring separately. All four demonstrated the same pattern for sons and daughters (Table 1), indicating that these loci are not on the sex chromosome. The nature of linkage was further calculated for the six families with at least 50 offspring. The recombination fraction ranged from 0.23 to 0.32 with an average of 0.27. The map distance between the melanic and the wingless locus was estimated following Haldane's map function (Haldane 1919). It ranged from 30.2 to 50.6 cM with an average value of 38.7 cM, nearly exactly the distance initially estimated from the group crossing.

Conclusion and discussion: using linkage in molecular studies

In conclusion, we have shown that the major loci for winglessness and melanism of the elytra are genetically linked on one of the nine autosomes (Smith 1953) of *A. bipunctata*. Knowledge of this linkage may facilitate future molecular studies on the genetic bases of these two traits in *A. bipunctata*, even though the loci are not closely linked. A candidate gene approach can attempt to identify these two loci in *A. bipunctata*. Developmental studies on *A. bipunctata* have suggested that genes involved in dorsoventral wing patterning provide candidates for winglessness (Lommen et al. 2009). Genetic tools, such as germ line transformation and RNA interference, are being developed for ladybirds (Kuwayama et al. 2006), and may further speed up such investigations.

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References

Bengtson SA, Hagen R (1977) Melanism in 2-spot ladybird *Adalia bipunctata* in relation to climate in Western Norway. Oikos 28(1):16–19. doi:10.2307/3543317

Brakefield PM (1984a) Ecological studies on the polymorphic ladybird *Adalia bipunctata* in The Netherlands. 1. Population biology and geographical variation of melanism. J Anim Ecol 53(3): 761–774. doi:10.2307/4658

Brakefield PM (1984b) Selection along clines in the ladybird *Adalia bipunctata* in The Netherlands—a general mating advantage to melanics and its consequences. Heredity 53:37–49

Brakefield PM (1985) Polymorphic Mullerian mimicry and interactions with thermal melanism in ladybirds and a soldier beetle—a hypothesis. Biol J Linn Soc 26(3):243–267. doi:10.1111/j.1095-8312.1985.tb01635.x

Brakefield PM, de Jong PW (2011) A steep cline in ladybird melanism has decayed over 25 years: a genetic response to climate change? Heredity 107(6):574–578. doi:10.1038/hdy.2011.49

Brakefield PM, Willmer PG (1985) The basis of thermal melanism in the ladybird *Adalia bipunctata*—differences in reflectance and thermal properties between the morphs. Heredity 54:9–14. doi:10.1038/hdy.1985.3

Creed ER (1975) Melanism in 2 spot ladybird—nature and intensity of selection. Proc R Soc Lond B Biol Sci 190(1098):135–148. doi:10.1098/rspb.1975.0083

De Jong PW, Gussekloo SWS, Brakefield PM (1996) Differences in thermal balance, body temperature and activity between non-melanic and melanic two-spot ladybird beetles (*Adalia bipunctata*) under controlled conditions. J Exp Biol 199(12):2655–2666

De Jong PW, Brakefield PM, Geerinck BP (1998) The effect of female mating history on sperm precedence in the two-spot ladybird, *Adalia bipunctata* (Coleoptera, Coccinellidae). Behav Ecol 9(6):559–565

Dobzhansky T (1924) Die geographische und individuelle Variabilitat von *Harmonia axyridis* Pall in ihren Wechselbeziehungen. Biologisches Zentralblatt 44:401–421

Dobzhansky T (1933) Geographical variation in lady-beetles. Am Nat 67:97–126

Haldane JBS (1919) The combination of linkage values, and the calculation of distances between the loci of linked factors. J Genet 8:299–309

Hammond PM (1985) Dimorphism of wings, wing-folding and wing-toiletry devices in the ladybird, *Rhyzobius litura* (F) (Coleoptera, Coccinellidae), with a discussion of inter-population variation in this and other wing-dimorphic beetle species. Biol J Linn Soc 24(1):15–33

Kuwayama H, Yaginuma T, Yamashita O, Niimi T (2006) Germ-line transformation and RNAi of the ladybird beetle, *Harmonia axyridis*. Insect Mol Biol 15(4):507–512. doi:10.1111/j.1365-2583.2006.00665.x

Lommen STE, De Jong PW, Brakefield PM (2005) Phenotypic plasticity of elytron length in wingless two-spot ladybird beetles, *Adalia bipunctata* (Coleoptera : Coceinellidae). Eur J Entomol 102(3):553–556

Lommen STE, Middendorp CW, Luijten CA, Van Schelt J, Brakefield PM, De Jong PW (2008) Natural flightless morphs of the ladybird beetle *Adalia bipunctata* improve biological control of aphids on single plants. Biol Control 47(3):340–346. doi:10.1016/j.biocontrol.2008.09.002

Lommen STE, Saenko SV, Tomoyasu Y, Brakefield PM (2009) Development of a wingless morph in the ladybird beetle, *Adalia bipunctata*. Evol Dev 11(3):278–289. doi:10.1111/j.1525-142X.2009.00330.x

Lommen STE, Holness TC, Van Kuik AJ, De Jong PW, Brakefield PM (2012) Releases of a natural flightless strain of the ladybird beetle *Adalia bipunctata* reduce aphid-born honeydew beneath urban lime trees. Biocontrol. doi:10.1007/s10526-012-9478-7

Lusis JJ (1961) On the biological meaning of colour polymorphism of lady-beetle *Adalia bipunctata* L. Latvijas Entomologs 4:3–29

Majerus MEN (1994) Ladybirds, vol 81. New Naturalist, Collins

Majerus MEN (1998) Melanism: evolution in action. Oxford University Press, Oxford

Majerus MEN, Zakharov IA (2000) Does thermal melanism maintain melanic polymorphism in the two-spot ladybird *Adalia bipunctata* (Coleoptera: Coccinellidae)? Zh Obshch Biol 61(4): 381–392

Majerus MEN, Odonald P, Kearns PWE, Ireland H (1986) Genetics and evolution of female choice. Nature 321(6066):164–167

Marples NM, De Jong PW, Ottenheim MM, Verhoog MD, Brakefield PM (1993) The inheritance of a wingless character in the 2-spot ladybird (*Adalia bipunctata*). Entomol Exp Appl 69(1):69–73

Michie LJ, Mallard F, Majerus MEN, Jiggins FM (2010) Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*. J Evol Biol 23(8):1699–1707. doi:10.1111/j.1420-9101.2010.02043.x

Michie LJ, Masson A, Ware RL, Jiggins FM (2011) Seasonal phenotypic plasticity: wild ladybirds are darker at cold temperatures. Evol Ecol 25(6):1259–1268. doi:10.1007/s10682-011-9476-8

Obrycki JJ, Harwood JD, Kring TJ, O'Neil RJ (2009) Aphidophagy by Coccinellidae: application of biological control in agroecosystems. Biol Control 51(2):244–254. doi:10.1016/j.biocontrol.2009.05.009

Pope RD (1977) Brachyptery and wing polymorphism among Coccinellidae (Coleoptera). Syst Entomol 2(1):59–66. doi:10.1111/j.1365-3113.1977.tb00361.x

Smith SG (1953) Chromosome numbers of Coleoptera. Heredity 7(1):31–48. doi:10.1038/hdy.1953.3

Timofeeff-Ressovsky NW (1940) Zur Analyse des Polymorphismus bei Adalia bipunctata L. Biologisches Zentralblatt 60:130–137

True JR (2003) Insect melanism: the molecules matter. Trends Ecol Evol 18(12):640–647. doi:10.1016/j.tree.2003.09.006

Ueno H, de Jong PW, Brakefield PM (2004) Genetic basis and fitness consequences of winglessness in the two-spot ladybird beetle, *Adalia bipunctata*. Heredity 93(3):283–289

Van 't Hof AE, Edmonds N, Dalikova M, Marec F, Saccheri IJ (2011) Industrial melanism in British peppered moths has a singular and recent mutational origin. Science 332(6032):958–960. doi:10.1126/science.1203043

Van't Hof AE, Saccheri IJ (2010) Industrial melanism in the peppered moth is not associated with genetic variation in canonical melanisation gene candidates. Plos One 5(5):e10889. doi:10.1371/journal.pone.0010889

Wittkopp PJ, Beldade P (2009) Development and evolution of insect pigmentation: genetic mechanisms and the potential consequences of pleiotropy. Semin Cell Dev Biol 20(1):65–71. doi:10.1016/j.semcdb.2008.10.002

Wittkopp PJ, Carroll SB, Kopp A (2003) Evolution in black and white: genetic control of pigment patterns in Drosophila. Trends Genet 19(9):495–504. doi:10.1016/s0168-9525(03)00194-x