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Genetics and selective breeding of variation in wing truncation in a flightless aphid control agent

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Abstract

Augmentative biological control by predaceous ladybird beetles can be improved by using flightless morphs, which have longer residence times on the host plants. The two-spot ladybird beetle, Adalia bipunctata (L.) (Coleoptera: Coccinellidae), is used for the biological control of aphids in greenhouses and on urban trees. Flightlessness due to truncated wings occurs at very low frequency in some natural populations of A. bipunctata. Pure-breeding strains of this ‘wingless’ genotype of A. bipunctata can easily be obtained in the laboratory. Such strains have not been commercialized yet due to concerns about their reduced fitness compared to wild-type strains, which renders mass production more expensive. Wingless strains exhibit, however, wide intra-population phenotypic variation in the extent of wing truncation which is related to fitness traits. We here use classical quantitative genetic techniques to study the heritability and genetic architecture of variation in wing truncation in a wingless strain of A. bipunctata. Split-families reared at one of two temperatures revealed strong family-by-temperature interaction: heritability was estimated as 0.64 ± 0.09 at 19 °C and 0.29 ± 0.06 at 29 °C. Artificial selection in opposite directions at 21 °C demonstrated that the degree of wing truncation can be altered within a few generations resulting in wingless phenotypes without any wing tissue (realized h² = 0.72), as well as those with minimal truncations (realized h² = 0.61) in two replicates. The latter lines produced more than twice as many individuals. This indicates that selective breeding of wing truncation may be exploited to improve mass rearing of flightless strains of A. bipunctata for commercial biological control. Our work illustrates that cryptic variation can also be a source for the selective breeding of natural enemies.

Introduction

The augmentative biological control of aphids (Hemiptera: Aphididae) with predaceous ladybird beetles (Coleoptera: Coccinellidae) has variable success. Failures of aphid control have been associated, among others, with the tendency of the adult beetles to fly away from the host plants soon after release, whether in greenhouses (Hämäläinen, 1977), urban trees (Brakefield, 1984), or outdoor crops (Kieckhefer & Olson, 1974). Reduced flight ability in predaceous ladybird beetles has therefore received the interest of researchers over decades (Lommen, 2013). Experimental work has shown that flightless morphs of several species, as obtained by diverse methods, have a longer residence time on host plants and can improve aphid control (Ignoffo et al., 1977; Ferran et al., 1998; Tourniaire et al., 1999; Weissenberger et al., 1999; Lommen et al., 2008; Seko et al., 2008; Adachi-Hagimori et al., 2011; Iguchi et al., 2012; but see Riddick, 2017). A flightless strain of Harmonia axyridis Pallas is commercially available in Japan (MAFF, 2014) but this species has recently been banned from commercial use outside its
native range due to its invasiveness (van Lenteren et al., 2008; Roy et al., 2016). Naturally occurring flightless morphs of *Adalia bipunctata* (L.) may provide another opportunity to improve augmented aphid control.

*Adalia bipunctata* is native to a large part of the Western world, including Europe. It has been commercially available for more than 25 years (van Lenteren, 2012), and has gained importance after the ban of *H. axyridis* (van Lenteren et al., 2008). Wild-type *A. bipunctata* are fully winged and monomorphic in their wing length, with elytra (fore wings) covering the entire dorsal surface of the abdomen (Figure 1), and the flight wings folded underneath. A few flightless morphs with truncated wings have been found in the wild, in at least one population in The Netherlands, and five in the UK (Majerus & Kearns, 1989; Marples et al., 1993; MEN Majerus, pers. comm.). Both their elytra and flight wings are truncated (Lommen et al., 2009), making them incapable of flight. Although they may have some wing tissue, we will call them – and the corresponding trait – ‘wingless’. The wingless trait is determined by a single major locus with the winged, wild-type allele being dominant over the wingless (Marples et al., 1993). Pure-breeding wingless strains of the wingless (homozygote recessive) genotype can therefore be easily obtained from outcrossing a single wild-collected individual carrying the wingless trait. Such wingless strains of *A. bipunctata* showed the same voracity towards aphids as their winged conspecifics (Lommen et al., 2008). They improved control of *Myzus persicae* Sulzer aphids on single pepper plants in experiments in the greenhouse (Lommen et al., 2008), and reduced the amount of honeydew underneath aphid-infested urban lime trees (Lommen et al., 2013).

Wingless *A. bipunctata* are not yet commercially available, however, because producers of natural enemies are concerned about their reduced fitness (J van Schelt, Koppert, pers. comm.). Ueno et al. (2004) showed that wingless morphs of *A. bipunctata* have a longer development time, a reduced life span, and a lower lifetime reproduction compared to their winged conspecifics. This renders mass production of wingless strains less economically viable than that of the wild-type. Pure wingless lines of *A. bipunctata*, however, exhibit wide variation in the extent of wing truncation which is associated with several fitness traits (Marples et al., 1993; Ueno et al., 2004; Lommen et al., 2009). Wingless individuals with milder truncations develop faster and have longer life spans (Ueno et al.,

| Major gene | G | aa | A- |
|------------|---|----|----|
| P          |   | ‘wingless’ | ‘winged’ |

| G | Unknown |
|---|---------|
| P | Variation released: continuous variable extent of wing truncation measured in ‘degree of winglessness’ (DWL) from 0 to 16 |
|   | Cryptic variation: wildtype |

![Figure 1](image_url) Genetic (G) and phenotypic (P) variation in wing morphology in *Adalia bipunctata*. The upper part of the graph covers the major gene regulating winglessness, the lower part shows the modifier genes regulating the extent of wing truncation in wingless morphs. This extent is expressed in the degree of winglessness of the elytra (DWL), a categorical measure with values ranging from 0 (no wing tissue) to 16 (elytra covering more than three quarters of the abdomen). The pictures of the six ‘wingless’ specimens represent extremes of particular DWL categories. Note that the middle two individuals are melanistic colour morphs, whereas the others are typical morphs. A wild-type (‘winged’) typical ladybird beetle is shown on the right. Genetic variation for wing truncation is not expressed in phenotypic variation in the wild-type, and is therefore cryptic.
2004) suggesting that they may perform better in mass production.

Earlier work indicates that variation in wing truncation in wingless *A. bipunctata* is partly under genetic control, and that it is also strongly affected by rearing temperature (Ueno et al., 2004; Lommen et al., 2005, 2009). A better understanding of the genetics is essential to assess the possibilities for selectively breeding flightless *A. bipunctata* with a specific degree of wing truncation. Here, we study the genetics of variation in wing truncation in a flightless strain of *A. bipunctata*. We specifically ask (1) what is the heritability of variation in wing truncation under different temperatures, and (2) can the extent of wing truncation be altered by artificial selection?

**Materials and methods**

**Wingless beetle stock population**

A laboratory stock of wingless *A. bipunctata* was established from hundreds of individuals collected from trees or shrubs in Utrecht in The Netherlands on several occasions in 2000–2004. Two of the collected individuals were found to carry copies of the wingless allele, one was a homozygote and the other a heterozygote. The wingless allele was fixed in the laboratory stock by outcrossing these beetles to more than 100 wild-type individuals and then selecting only wingless phenotypes from the F2 for further breeding. The wingless stock was maintained in a climate cabinet at 20.5 ± 1.5 °C, 65 ± 5% r.h., and L16:D8 photoperiod. Larvae and adults were fed *Ephestia kuehniella* Zeller eggs, and adults received a supplement of flower pollen. This stock was then used for all experiments between 2005 and 2007.

**Measuring wing truncation**

Variation in wing truncation in wingless *A. bipunctata* strains is continuous from individuals lacking all wing tissue to ones missing only very small pieces at the wing tips (Figure 1). It results from differential rates of wing development in the larval stages, which is regulated by both genetic and environmental factors (Ueno et al., 2004; Lommen et al., 2005, 2009). We used the measure ‘degree of winglessness’ (DWL) to quantify the wing truncation of wingless *A. bipunctata* (see also Lommen et al., 2005). The degree of the truncation of the elytra is closely correlated with that of the flight wings (Lommen et al., 2009). The latter are not visible when folded beneath the elytra and, therefore, DWL is based on the length of the elytra alone. DWL indicates the length of the truncated elytra relative to those of wild-types covering the entire abdomen. Because the left and the right elytron, and even the medial and outer half of a single elytron, may be truncated to different extents (Figure 1), the length of each of these four halves is scored separately. The length of each of the four elytral sections was estimated by eye and assigned to one of the following classes: 0 (with no tissue visible), 1 (only a small bud, not extending beyond a quarter of the maximum elytron length), 2 (more than a quarter and up to half of the maximum), 3 (more than half and up to three quarters of the maximum), or 4 (more than three quarters of the maximum). The sum of these four scores is an individual’s DWL, with a minimum value of 0 and a maximum of 16, with higher numbers corresponding to increased elytron length and thus, milder truncations (Figure 1). This method is highly repeatable (Lommen et al., 2005).

**Parent–offspring regression experiment**

Various families of wingless *A. bipunctata* were bred to estimate the heritability of DWL by parent–offspring regression. The offspring of each family was split and reared at two temperatures to examine environmental effects and potential gene-by-environment interactions. Specifically, we set up 49 breeding pairs of virgin, 3-week-old adults from the wingless stock in the rearing facility. The DWL values of the male and the female within a pair were equal, except in eight pairs where they differed by 1 DWL, and in one pair where they differed by 2 DWL. In contrast, we maximized variation in the DWL values among couples (mid-parent values ranged from 0 to 15) to increase the reliability of the heritability estimate (Falconer & Mackay, 1996). Pairs were kept in round Petri dishes (55 mm diameter) with paper lined in the cover to stimulate egg-laying. Papers were collected and replaced every 2–3 days over a period of 12 days. First-instar larvae which had hatched from the collected papers were reared in groups of up to 10 in Petri dishes until moulting into L2. Histological examination showed that the formation of wing structures does not begin until this larval stage or later (Lommen et al., 2009). These second instars were randomly assigned to an incubator with one of two rearing temperatures, 19 or 29 °C, which had earlier shown large disparity in wing development (Lommen et al., 2005). The position of the larvae within the incubators was changed regularly to avoid potential effects of variation in microclimates. The sex and DWL of the emerged adults were determined. Only families with at least five offspring at each rearing temperature were used in the statistical analysis (n = 41 families, mid-parent values from 0 to 12, median number of offspring was 15 at 19 °C and 12 at 29 °C). Statistical analysis used the package R v.2.6.1 (R Development Core Team, 2007). The mid-offspring values were represented by the median DWL of each family to correct for non-normal frequency distributions. We pooled sons and daughters as sample sizes per sex were small whereas
the variation among offspring was large, and because earlier work revealed no differences between the sexes (Lommen et al., 2005). The heritability experiment was analysed using linear models fitted by least square methods using weighting by the number of offspring. In the full model, the mid-offspring value of DWL was used as a response variable, and mid-parent value of DWL, temperature, and their interaction term were specified as fixed factors. We tested the significance of the interaction by removing this term from the model and comparing the models by an F-test. The heritability of DWL was then estimated for each rearing temperature separately as the mid-offspring on mid-parent regression.

Artificial selection experiment

An artificial selection experiment was performed to estimate the realized heritability of DWL in our wingless stock at 20.5 °C and 65% r.h. We selected in both upward and downward directions using two replicates. Generation 0 (G0) was derived from the wingless stock after artificial selection for increased fecundity over 10 generations (STE Lommen, KG Koops, PW de Jong & PM Brakefield, unpubl. data). We randomly split this population in half to create the two replicate lines: ‘C’ (original n = 1 136 virgin beetles) and ‘D’ (n = 1 126). For each replicate, we selected 60 males and 60 females with the most extreme values in each direction: ‘L’ and ‘H’ for downward and upward selection, respectively (yielding lines ‘CL’, ‘CH’, ‘DL’, and ‘DH’; Table 1). For each line, the selected adults were randomly distributed into three breeding groups, each of 20 males and 20 females, at an age of 6–26 days when most beetles are sexually active (Hemptinne et al., 2001). Breeding groups were kept in Petri dishes (12 × 12 cm) with paper folded like a harmonica to stimulate egg-laying. Eggs were collected every 2–3 days over 2 weeks (except for G4, where eggs were collected over 4 weeks) and reared to adulthood. Newly emerged adults were collected every 2–3 days and housed individually in small dishes (55 mm diameter) until after determination of their sex and DWL to ensure that all adults remained virgin until selection. The selection was continued the same way for each line (selecting the 60 males and 60 females with most extreme values) for four generations. When insufficient offspring were bred, however, fewer than 60 adults per sex were collected (G2 and G3 of line CL; Table 1). When the most extreme DWL value in the direction of selection contained more than 60 adults per sex, in contrast, all of them were selected as parents (Table 1). The number of parents selected and the selection differential thus varied among lines and generations (Table 1). We performed an additional generation of selection for the upward selected lines to check whether the phenotype could be further selected toward an elytral phenotype closer to that of the wild-type. This required a more

| Line | Generation |
|------|------------|
|      | 0          | 1    | 2     | 3     | 4     | 5     |
| CL   | Bred       | 4 (1136) | 1 (464) | 0 (42) | 0 (35) | 0 (156) | –     |
|      | Selected   | 0 (120) | 0 (133) | 0 (39) | 0 (32) | –     | –     |
| CH   | Bred       | 4 (1136) | 8 (552) | 8 (785) | 13 (606) | 16 (519) | 16 (881) |
|      | Selected   | 9 (120) | 12 (120) | 16 (120) | 16 (160) | 16 (120) | –     |
| DL   | Bred       | 4 (1126) | 2 (329) | 0 (292) | 0 (133) | 0 (99) | –     |
|      | Selected   | 0 (120) | 0 (122) | 0 (149) | 0 (102) | –     | –     |
| DH   | Bred       | 4 (1126) | 8 (710) | 9 (455) | 12 (547) | 16 (777) | 16 (781) |
|      | Selected   | 9 (120) | 12 (119) | 14 (120) | 16 (160) | 16 (120) | –     |

1CL and CH were both selected from the C-group of G0 adults.
2For a subset of 320 beetles, elytra of those with DWL = 16 were described in more detail.
3DL and DH were both selected from the D-group of G0 adults.
4For a subset of 577 beetles, elytra of those with DWL = 16 were described in more detail.
detailed classification of the extent of elytral truncation for beetles with the maximum DWL value (16). For beetles with a DWL of 16 in the G4 and G5 of the upwards selected lines, we therefore recorded whether the elytra covered the full length of the abdomen or not. Only beetles with elytra covering the full abdomen were selected for the additional round of selection.

To analyze the results, we first tested whether DWL differed between the sexes for each selection line at each generation. We used a Wilcoxon Signed Rank Test with continuity correction, adjusting the significance threshold \( \alpha \) with a Holm-Bonferroni correction. As no significant differences were detected, we pooled the sexes for further analysis. A comparable procedure was followed to examine differences in DWL between the low and high line of each selection. We then estimated the realized heritability of the DWL for each selection line as the slope of the regression of the cumulative response to selection (median offspring value – median of the total parental population) on the cumulative selection differential (median of the selected parents – median of the total parental population). We calculated the mean realized heritability for each direction of selection by averaging the values of the two replicates. A heterogeneity G-test (Sokal & Rohlf, 1995) was used to test whether the proportion of beetles with a DWL of 16, and with elytra covering the full length of the abdomen, had changed after the extra round of selection in the upward selection lines.

Data archiving
The data sets corresponding to the above experiments are archived in the open data repository OSF (https://osf.io/wmaq8/).

Results

Parent–offspring regression experiment
A total of 41 pairs produced sufficient offspring to be included in the analysis, and their mid-parent DWL ranged from 0 to 12. There was a significant interaction between mid-parent value of DWL and temperature \( (F = 9.98, \text{d.f.} = 1, P<0.01) \). Raising larvae at the highest temperatures usually yielded lower median DWL values (Figure 2), with this pattern being observed in 35 of 41 families. The mean (± SE) heritability was estimated as 0.64 ± 0.09 at 19 °C \( (F_{1,39} = 53.35, P<0.001, \text{adjusted } R^2 = 0.57) \) and 0.29 ± 0.06 at 29 °C \( (F_{1,39} = 23.00, P<0.001, \text{adjusted } R^2 = 0.35) \) (Figure 2).

Artificial selection experiment
Males and females of G0 did not significantly differ in DWL \( (n_{\text{males}} = 1130, n_{\text{females}} = 1132, W = 615508.5, P = 0.12) \), and no effect of sex was found in any of the generations of all four selection lines (e.g., for G1, CL: \( W = 28037.5, P = 0.34 \); CH: \( W = 35214, P = 0.36 \); DL: \( W = 12799, P = 0.38 \); DH: \( W = 67930, P = 0.070 \)). We observed a rapid response in DWL to artificial selection in the replicate lines in both directions (Figure 3). The upward and downward lines differed significantly in DWL in each replicate after only a single generation of selection (C: \( W = 17831.5 \); D: \( W = 13480 \), both \( P<0.001 \)). The response to selection continued until in both downward selected lines the median DWL reached the minimum possible value (0) after only two generations, whereas that of the upward selected lines both took four generations to reach the maximum score (16). Corresponding realized heritabilities were 0.72 ± 0.07 and 0.61 ± 0.03 for downward and upward selection, respectively (Figure 4).

Although the majority of the beetles in the upward direction had reached the maximum score in G4 (CH: 65%, DH: 50%), the percentage of individuals with elytra covering the full length of the abdomen was only 48 and 35% in CH and DH, respectively. After additional selection on parents with elytra covering the full length of the abdomen, the percentage of individuals with a DWL of 16 had increased in both replicates (CH: from 65 to 70%, \( G_{14} = 3.845, P<0.05 \); DH: from 50 to 71%, \( G_{14} = 68.216, P<0.001 \)), but the percentage with wings covering the full abdomen, had changed after the extra round of selection in the upward selection lines.

![Figure 2](https://openrepository.com/image.png)

**Figure 2** Heritability of wing truncation in flightless Adalia bipunctata. The degree of winglessness (DWL), with lower values corresponding to more severe wing truncations, is plotted as the midoffspring on midparent values for split families raised at 19 or 29 °C. The slope of the regression lines (shown between 95% confidence intervals) indicates the heritability \( (h^2) \) of DWL estimated for each temperature, and differs significantly between the two temperatures.
abdomen had only increased in line DH (CH: from 48 to 43%, $G_H = 2.297$, $P = 0.13$; DH: from 35 to 46%, $G_H = 16.493$, $P < 0.001$). It is noteworthy that the majority of these beetles remained distinct from wild-types because of irregularities in the three-dimensional shape of the elytra.

The number of ladybirds in the upward selected lines was much higher than in the corresponding downward selected lines in each generation in both replicate lines (Table 1; note that this cannot be caused by the 40 additional adults selected in the upward lines in G3 alone).

**Discussion**

**Selective breeding of wingless *Adalia bipunctata***

Our results show that the extent of wing truncation in wingless strains of *A. bipunctata* is highly heritable, and can quickly be altered by selective breeding. Wingless
phenoype with little variation in wing truncations were obtained in each direction of selection. Thus, selection downwards resulted in individuals without any wing tissue, and selection upwards in individuals with only very small truncations in wings. This demonstrates a practical way to breed strains of wingless *Adalia bipunctata* with mild wing truncations. The strong gene-by-environment interactions found suggest that the response to selection can be optimized by choosing conditions in which heritability is high. Our artificial selection experiment is in line with earlier findings that fitness traits improve with decreasing degrees of truncation if necessary. Another risk of continued selective breeding of wingless *Adalia bipunctata* with minor wing truncations is the potential restoration of the wild-type phenotype, including its flight ability. Histological examination of the flight muscles of wingless morphs revealed no reduction in muscle tissue (SV Saenko & STE Lommen, unpubl. results), and wingless individuals were frequently observed to move their truncated wings up and down in a similar manner as wild-types (STE Lommen & KG Koops, unpubl. obs.). This suggests that the truncation of the wings itself impairs flight. The flight wings of wingless individuals were usually reduced or malformed (Lommen et al., 2009), and the beetles were typically incapable of directed flight. However, observations on flight behaviour suggest that the potential for at least some flight activity was restored in a small proportion of wingless individuals after artificial selection in an upward direction (STE Lommen, KG Koops, PW de Jong & PM Brakefield, unpubl. data; File S1). In the artificial selection experiment, an extra round of selection in the upward direction resulted in an increased proportion of individuals in the highest DWL category and, in a single line, in more wingless beetles with elytra covering the full abdomen. This suggests that at least one polymorphic modifier locus had not yet reached fixation, and that further selection could result in phenotypes even closer to the wild-type. The potential risk of full restoration of flight should therefore be taken into account.

**Risks of releasing selected wingless Adalia bipunctata**

As the degree of wing truncation in *A. bipunctata* is partially determined by temperature, offspring of released wingless *A. bipunctata* may differ in this degree from their parents, depending on the environmental temperature. After release of large numbers of wingless *A. bipunctata* for biological control, they will likely mate with conspecifics from wild populations. As no heterosis was found and

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**Figure 4** Realized heritabilities ($h^2$) of wing truncation in an artificial selection experiment of flightless *Adalia bipunctata*. Cumulative response to artificial selection is plotted as a function of the cumulative selection differential for both replicates (C, D) selected in upward (H) and downward (L) directions for degree of winglessness (DWL). The dashed horizontal line represents the median starting value of all selection lines, corresponding to DWL = 4. The mean realized heritabilities (thick solid lines) are given for both directions of selection.
wingless morphs have a disadvantage in many fitness traits (Ueno et al., 2004; Lommen, 2013) we expect the wingless allele to be selected against by natural selection, and not reach high frequencies in natural populations.

The genetic architecture of wing length variation in Adalia bipunctata

The rapid response to artificial selection suggests that only a few loci and alleles of large effect are involved in determining a very substantial proportion of the cryptic variation in the extent of wing truncation in wingless A. bipunctata. A pedigree analysis of four generations of families derived from the selection lines indicates that the inheritance of the extent of wing truncation in our wingless A. bipunctata stock is polygenic, and that these genes are likely to be multi-allelic (see File S2). None of the experiments indicates genetic linkage between these genes and sex. Thus, we can now present a more complete model of the genetics of winglessness in this species (Table 2).

| Regulating | Alternative alleles | Position | Heritability | Expression | Source |
|------------|---------------------|----------|--------------|------------|--------|
| Major gene | Winglessness        | 1, 2     | On an autosome, linked with gene for melanism | 1.0        | Marples et al., 1993; Lommen et al., 2012 |
| Modifier genes | Extent of wing truncation | >2 >2 at each locus | On autosomes | Parent-offspring regression (parents at 21 °C):
| | | | | 1. Offspring at 19 °C:
| | | | | 0.64 ± 0.09
| | | | | 2. Offspring at 29 °C:
| | | | | 0.29 ± 0.06
| | | | | Realized $h^2$ (at 21°C):
| | | | | 1. Upward: 0.61 ± 0.03
| | | | | 2. Downward: 0.72 ± 0.07

This is likely to involve at least three polymorphic loci on the autosomes regulating wing morphology: one determining the wingless status (Marples et al., 1993; upper part of Table 2) linked to the locus for melanism (Lommen et al., 2012), and at least two multi-allelic modifier loci that are affected by the environment and regulate the cryptic variation in the extent of wing truncation within the wingless phenotype (this paper; lower part of Table 2).

Selecting on cryptic variation

‘Cryptic variation’ is standing genetic variation that does not contribute to the range of phenotypes observed under standard conditions that natural populations experience, but can be ‘released’ and contribute to the phenotype under perturbing environmental or genetic conditions. The genetic variation for the extent of wing truncation in A. bipunctata is cryptic, as it stems from standing genetic variation in the wild, but does not result in phenotypic

Table 2 A genetic model suggested for natural wing polymorphism in Adalia bipunctata. The table shows the number of genes involved (n), the number of alternative alleles per gene, the position of the genes, the heritability, and the expression of the trait regulated. The upper part of the table provides details on the major gene regulating winglessness, the lower part on modifier genes associated with variation in the expression of this trait.
variation in wing length in the wild-types. The existence of such cryptic variation has long been recognized (reviewed by Schlichting, 2008), and is common (e.g., Rutherford & Lindquist, 1998; Sangster et al., 2008). It has regained attention in the past decade in relation to the evolvability of traits (Le Rouzic & Carlberg, 2008; McGuigan & Sgro, 2009; Hayden et al., 2011). To our knowledge, however, cryptic variation has not been exploited yet in selective breeding of biocontrol agents. Our work illustrates that such cryptic variation may also be a source for the selective breeding of natural enemies.

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Selective breeding of ladybird wing truncation

Additional Supporting Information may be found in the online version of this article:

Figure S1. Pedigrees established from beetles artificially selected for extreme degrees of winglessness

File S1. Data on flight ability of 'wingless' Adalia bipunctata.

File S2. Pedigree analysis of degree of wing truncation in 'wingless' Adalia bipunctata.