

Temporal stability of morph frequency in central European populations of *Adalia bipunctata* and *A. decempunctata* (Coleoptera: Coccinellidae)

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Abstract. In central Europe *Adalia bipunctata* (L.) occurs in two main colour morphs (typical, melanic), and *A. decempunctata* (L.) occurs in 3 morphs (spotted, chequered, dark). Temporal variation in the relative frequency of morphs was recorded in populations of the Czech Republic where geographic variation in morph frequency is low. Seasonal trends were investigated in samples collected by a light-trap run daily from March to November for 14 years. In *A. bipunctata* the melanic form was more abundant in autumn than in spring but the difference was not significant. In *A. decempunctata* morph proportions did not change seasonally. Samples were also collected by sweepnet from stands of many plant species. In both *Adalia* species the morph proportions did not differ significantly among collections made on different plants. Long-term changes in morph proportions were analysed by pooling annual samples over all host plants. In *A. bipunctata*, sampled in 15 years between 1971–2004, there was no significant change in proportion of typical (90.1%) and melanic (9.9%) forms. In *A. decempunctata*, sampled in 12 years between 1976–2004, the proportions of “spotted” (mean over the years 29.4%), “chequered” (42.2%) and “dark” (21.3%) morphs varied between years. There was a trend toward an increasing proportion of the spotted form in the 2000s compared to the 1970s and 1980s.

INTRODUCTION

The enormous colour variation that occurs within some coccinellid species has attracted much research attention. Variability of adult coloration typically consists of particular patterns of head, pronotum and elytra colour and minor differences in the number, size and shape of elytral spots. An important domain of investigation in this diversity is variation in proportion of colour morphs in local populations. These studies were pioneered by Dobzhansky (1933) who showed the relationship between climate and coloration in several coccinellid species. The morphs of each species were ranked according to the proportion of body surface that is dark, starting from the light (non-melanic) forms to the dark (melanic) forms. Data from Palearctic species revealed the prevalence of dark morphs in populations from humid and/or cold geographic areas and the dominance of light morphs in warm and dry areas.

Adalia bipunctata (L.) and *A. decempunctata* (L.) are examples of coccinellid species where colour polymorphism has been investigated extensively. Many studies published after 1900 addressed mechanisms that constrain the vast potential variation to a small number of realised morphs, the inheritance of morphs, the variation in morph proportions in natural populations, and their likely significance for survival (reviews: Hodek & Honěk, 1996; Majerus & Zakharov, 2000). *Adalia* species attracted wide attention because of the heritable nature of morphs in this genus (Schröder, 1909; Fiori, 1928; Lusi, 1932). Adults were collected in many localities and causes of the differences in morph proportions between local samples were hypothesized. Large differences in morph proportions were observed between local *A. bipunctata* popula-

tions from Mediterranean (Scali & Creed, 1975) and Atlantic regions of western and northern Europe (e.g. Creed, 1971; Bengtson & Hagen, 1977), particularly in urban areas (Zakharov, 2003). These studies identified several climatic factors that may select for local differences in morph proportions. The most important cause of differences in morph proportions is the duration of sunshine during the period of breeding activity (Benham et al., 1974). In both *Adalia* species, aposematic function of morphs and differential predation were also proposed to explain differences in proportion of morphs (Brakefield, 1985). Temporal variation in morph proportions was revealed in parallel with geographic differences, becoming manifest as seasonal changes of morph proportions (Timofeeff-Ressovsky, 1940) or long term trends observed over large geographic areas (Brakefield & Lees, 1987). Similar mechanisms were proposed to explain temporal variation as for geographic differences (Lusi, 1961; Muggleton et al., 1975).

In contrast to the well established local variation in coastal western and northern Europe, the variation in morph proportions among local populations of *A. bipunctata* recently established in central Europe was small (Honěk, 1975; Klausnitzer & Schummer, 1983). The low proportion of melanic forms (<20%) observed in local populations since the 1970s contrasted with high proportions (>50%) of melanic forms recorded in this area in the early 20th century (Schröder 1901, 1902; Meissner, 1907a,b; Dobzhansky, 1924). Explaining this difference requires knowledge about temporal variation in morph frequencies.

Our 30-year record of morph frequencies in *A. bipunctata* and *A. decempunctata* populations of the Czech Republic enabled rigorous testing of seasonal and long

term variation in morph proportions. This was done in three steps. (i) Using data from a standard light-trap we tested seasonal variation in morph frequency. Nocturnal flight is likely not affected by differences in activity which may result from the differential thermoregulation of non-melanic and melanic morphs during the daytime. As seasonal changes in morph proportions were not significant, sweepnet samples (collected mainly in June–August) were used to test (ii) the differences between samples from different host plant/aphid associations. After demonstrating their similarity, we tested (iii) for each *Adalia* species variation between years using data of all host plant/aphid associations pooled over the seasons, in the period between 1971–2004.

MATERIAL AND METHODS

Sampling

Light trap. Flying *Adalia* adults were sampled with a light trap run for 14 years between 1973–1989. The trap (Novák, 1983) consisted of 250 W mercury vapour lamp (luminosity 1200 lm, about 25% UV light) with a 1 × 1.2 m white panel in the background. A grid of thin wires stretched 7 mm apart was placed before the white panel and charged with a 2000 V 2 mA electric current. The insects flying to the light source were knocked down and fell to a bottle containing chloroform vapour. The trap, placed 8 m above the ground at the southern wall of a building, faced a garden sparsely planted with ornamental trees. Every year the trap was run daily, from sunset to sunrise, from March to November. Numbers of captured *Adalia* were recorded daily.

Sweepnet collections. *Adalia* adults were always collected by the same person (A.H.) sweeping with a standard sweepnet (30 cm diameter, handle 90 cm long). Sampling took place during the daytime from crops, wild herbaceous plants, shrubs and trees, from the ground level up to ca. 2 m height. A “sample” refers to a series of *Adalia* adults collected from one plant stand on a particular day, each consisting of 50–500 sweeps. Only samples including at least one *Adalia* individual were considered.

Morph classification

Adults of both species were classified according to the colour pattern of the elytron. *A. bipunctata* adults were divided into (i) “typical” (light, non-melanic) form with one black spot on red background at each elytron, and (ii) “melanic” (dark) form with two or three red spots on the black background of each elytron. In *A. decempunctata* we distinguished three morphs referred to as (i) “spotted” form with 0 to 5 dark spots on yellowish to reddish background of each elytron, (ii) “chequered” form with yellowish to reddish spots on brown to black background of each elytron, and (iii) “dark” form with one sickle-shaped yellowish to reddish spot at the shoulders on brown to black background of each elytron. Minor variations in the size and shape of the spot in the typical morph of *A. bipunctata*, and in the number of spots on the elytron of spotted form of *A. decempunctata* were not evaluated.

Data processing

Mean proportions of morphs for particular months in the light-trap samples, and for particular host plants or years in sweepnet samples, were calculated after pooling original samples. The chi-square test or Fishers exact test (when numbers of adults of particular morphs were <10) were used to test the differences between months, host plant stands, and years. To test

for monotonic long-term changes in morph frequencies we used regression of morph proportion on time (month, year). As the data were proportions, General Linear Models with the binomial family (GLM-b) were used to perform a weighted logistic regression. If the data showed over- or under-dispersion, quasibinomial family (GLM-qb) was used (Crawley, 2002). The analysis was done within the R environment (R Development Core Team, 2004).

RESULTS

Seasonal variation

Adalia bipunctata (Table 1) was captured from March to November. The overall proportion of the melanic morph in light trap samples was 9.8%, but proportions in particular months varied between 0–20%. Overall, the differences in melanic frequency among samples across months were not significant (chi square = 6.16, df = 17, $p > 0.05$). We therefore tested the difference between pooled autumn (before hibernation) and spring (after hibernation) samples, the latter presumably reflecting winter mortality of morphs. Comparison of the combined October plus November and March plus April samples showed that melanic form tended to be more abundant in the autumn (18%) than in the spring (5%), although the difference was not significant (Fishers exact test, $p = 0.27$). When May data were included in the spring sample the proportion of melanics was further decreased (3%), but the difference still failed to be significant ($p = 0.09$). The proportion of melanics in June–September, when most sweepnet data were collected, was intermediate, between 6.1–10.5%. The proportion of melanic forms increased from May to November but this increase was not significant (GLM-qb, $p = 0.09$). Flying *A. decempunctata* were captured in May–September (Table 2). The overall proportions of spotted, chequered and dark morphs were 31.2, 46.0 and 22.8%, respectively. Although the proportions of particular morphs varied among months, the differences were not significant (chi square = 7.35, df = 14, $p > 0.05$). There was no seasonal

TABLE 1. Numbers (n) and proportions (%) of adults of typical and melanic forms of *A. bipunctata* flying to the light trap at Praha-Ruzyně over 14 sampling years between 1973–1989. Samples indicate the number of years the species was captured.

	Samples n	Form		Total n
		typical n (%)	melanic n (%)	
March	3	11 (91.7)	1 (8.3)	12
April	6	10 (100.0)	0 (0.0)	10
May	5	14 (100.0)	0 (0.0)	14
June	11	31 (93.9)	2 (6.1)	33
July	13	152 (89.9)	17 (10.1)	169
August	13	145 (89.5)	17 (10.5)	162
September	10	60 (92.3)	5 (7.7)	65
October	12	47 (85.5)	8 (14.5)	55
November	5	8 (80.0)	2 (20.0)	10
Total	78	478 (90.2)	52 (9.8)	530

TABLE 2. Numbers (n) and proportions (%) of adults of spotted, chequered and dark forms of *A. decempunctata* flying to the light trap at Praha-Ruzyně. Samples indicate the number of years the species was captured.

	Samples n	Form			Total n
		spotted n (%)	chequered n (%)	dark n (%)	
May	6	7 (50.0)	5 (35.7)	2 (14.3)	14
June	12	11 (29.7)	18 (48.6)	8 (21.6)	37
July	11	61 (28.1)	107 (49.3)	49 (22.6)	217
August	11	44 (35.8)	48 (39.0)	31 (25.2)	123
September	8	3 (23.1)	8 (61.5)	2 (15.4)	13
Total	48	126 (31.2)	186 (46.0)	92 (22.8)	404

trend in changes of morph proportions (GLM-b, $p > 0.55$).

Variation between host plants

Since the sweepnet samples were taken from host plants populated by typical aphid species, but also from stands without aphids, we categorized the results according to host plant only. In *A. bipunctata*, the average proportions of typical (90.1%) and melanic (9.9%) forms in all 566 samples from 46 host plant species (Table 3) was similar to those from light trap samples. The differences between samples of the 16 most represented host plant species (≥ 7 samples available) was not significant (chi square = 17.26, $df = 31$, $p > 0.05$). The differences in proportion of morphs in pooled samples of tree hosts (3176 typical individuals, 355 melanics) and herb hosts (2702 typical, 288 melanics) also did not differ significantly (chi square = 0.32, $df = 3$, $p > 0.05$). In *A. decempunctata*, we collected only 252 samples from 35 host plant species (Table 4). In the total sample, morph proportions were 29.4% spotted, 49.2% chequered and 21.3% melanic. The variation in morph proportion among the 10 most represented host plants (≥ 5 samples available) was not significant (chi square = 22.7, $df = 29$, $p > 0.05$). Pooled samples of tree hosts (427 spotted, 722 chequered, 316 dark) and herb hosts (20 spotted, 25 chequered, 8 dark) were also not significantly different (chi square = 2.33, $df = 5$, $p > 0.05$). Since we found no differences in morph proportions among samples collected from particular host plants, samples of all host plants taken in a particular year were pooled for testing the annual differences.

Variation between years

Annual proportions of typical and melanic forms in *A. bipunctata* were established in 15 years between 1971–2004 (Table 3). Proportions of melanics varied between 6.7–14.5% in years when ≥ 10 individuals were captured. The differences between years were not significant (chi square = 16.7, $df = 29$, $p > 0.05$) and there was no long-term trend for increasing or decreasing proportions of the melanic morph (GLM-b, $p = 0.39$). Proportions of *A. decempunctata* morphs were established in 12 years between 1976 and 2004 (Table 4). Overall, the variation among annual samples was not significant (chi

TABLE 3. Numbers (n) and proportions (%) of typical and melanic forms of *A. bipunctata* collected on particular plants (1976–2004) and pooled within years (1971–2004). In 1971–1974 (data from Honěk, 1975) samples at particular localities were collected from different host plants and therefore not shown in the upper section of the Table. Host plant data is shown only for species with at least 5 samples.

Host plant	Samples n	Form		Total n
		typical n (%)	melanic n (%)	
<i>Acer</i> spp.	12	94 (90.4)	10 (9.6)	104
<i>Anthriscus sylvestris</i>	5	18 (90.0)	2 (10.0)	20
<i>Arctium</i> spp.	6	32 (82.1)	7 (17.9)	39
<i>Artemisia vulgaris</i>	54	613 (88.1)	83 (11.9)	696
<i>Betula pendula</i>	83	869 (92.0)	76 (8.0)	945
<i>Hordeum vulgare</i>	6	7 (100.0)	0 (0.0)	7
<i>Malus domestica</i>	5	39 (83.0)	8 (17.0)	47
<i>Matricaria inodora</i>	40	503 (91.5)	47 (8.5)	550
<i>Medicago sativa</i>	20	30 (90.9)	3 (9.1)	33
<i>Populus nigra</i>	5	44 (84.6)	8 (15.4)	52
<i>Prunus cerasus</i>	45	511 (89.2)	62 (10.8)	573
<i>Prunus domestica</i>	17	335 (89.3)	40 (10.7)	375
<i>Prunus padus</i>	25	322 (90.2)	35 (9.8)	357
<i>Pyrus communis</i>	5	18 (94.7)	1 (5.3)	19
<i>Quercus</i> spp.	7	17 (100.0)	0 (0.0)	17
<i>Salix</i> spp.	7	38 (88.4)	5 (11.6)	43
<i>Sambucus nigra</i>	9	72 (92.3)	6 (7.7)	78
<i>Tilia</i> spp.	70	770 (89.1)	94 (10.9)	864
<i>Triticum aestivum</i>	8	14 (93.3)	1 (6.7)	15
<i>Urtica dioica</i>	76	1184 (91.1)	115 (8.9)	1299
<i>Vicia faba</i>	15	104 (93.7)	7 (6.3)	111
<i>Zea mays</i>	6	23 (92.0)	2 (8.0)	25
Others ^a	40	221 (87.7)	31 (12.3)	252
Total	566	5878 (90.1)	643 (9.9)	6521
Year				
1971	3	248 (85.5)	42 (14.5)	290
1973	9	375 (90.1)	41 (9.9)	416
1974	16	1792 (90.4)	191 (9.6)	1983
1976	77	702 (88.6)	90 (11.4)	792
1977	109	1661 (90.5)	175 (9.5)	1836
1978	83	1354 (91.0)	134 (9.0)	1488
1979	100	945 (88.9)	118 (11.1)	1063
1980	48	446 (90.5)	47 (9.5)	493
1981	32	145 (92.4)	12 (7.6)	157
1982	33	196 (93.3)	14 (6.7)	210
1983	35	237 (89.4)	28 (10.6)	265
1986	9	55 (87.3)	8 (12.7)	63
2002	28	144 (90.6)	15 (9.4)	159
2003	2	3 (100.0)	0 (0.0)	3
2004	13	16 (88.9)	2 (11.1)	18
Total	597	8319 (90.1)	917 (9.9)	9236

^a Twenty three host plant species represented by ≤ 4 samples.

TABLE 4. Numbers (n) and proportions (%) of spotted, chequered and dark forms of *A. decempunctata* collected at particular plants and pooled within years. Host plant data is shown only for species with at least 3 samples.

	Samples n	Form			Total n
		spotted n (%)	chequered n (%)	dark n (%)	
Host plant					
<i>Acer</i> spp.	12	21 (29.2)	36 (50.0)	15 (20.8)	72
<i>Artemisia vulgaris</i>	6	5 (55.6)	3 (33.3)	1 (11.1)	9
<i>Betula pendula</i>	55	71 (32.3)	98 (44.5)	51 (23.2)	220
<i>Carpinus betulus</i>	5	5 (26.3)	8 (42.1)	6 (31.6)	19
<i>Corylus avellana</i>	3	4 (23.5)	9 (52.9)	4 (23.5)	17
<i>Fagus sylvatica</i>	3	2 (18.2)	5 (45.5)	4 (36.4)	11
<i>Matricaria inodora</i>	4	0 (0.0)	3 (75.0)	1 (25.0)	4
<i>Populus nigra</i>	5	0 (0.0)	6 (75.0)	2 (25.0)	8
<i>Prunus cerasus</i>	20	15 (28.8)	29 (55.8)	8 (15.4)	52
<i>Prunus domestica</i>	9	4 (16.7)	14 (58.3)	6 (25.0)	24
<i>Prunus padus</i>	18	36 (30.3)	58 (48.7)	25 (21.0)	119
<i>Quercus</i> spp.	13	17 (30.4)	23 (41.1)	16 (28.6)	56
<i>Salix</i> spp.	3	2 (28.6)	5 (71.4)	0 (0.0)	7
<i>Sambucus nigra</i>	3	1 (20.0)	2 (40.0)	2 (40.0)	5
<i>Tilia</i> spp.	62	244 (29.0)	421 (50.1)	175 (20.8)	840
<i>Urtica dioica</i>	12	10 (52.6)	9 (47.4)	0 (0.0)	19
Others ^a	19	10 (27.8)	18 (50.0)	8 (22.2)	36
Year					
1976	53	138 (30.7)	207 (46.1)	104 (23.2)	449
1977	21	19 (21.3)	51 (57.3)	19 (21.3)	89
1978	45	103 (24.9)	227 (54.8)	84 (20.3)	414
1979	28	24 (32.9)	39 (53.4)	10 (13.7)	73
1980	23	30 (27.3)	49 (44.5)	31 (28.2)	110
1981	10	7 (25.9)	15 (55.6)	5 (18.5)	27
1982	16	6 (23.1)	16 (61.5)	4 (15.4)	26
1983	22	22 (32.8)	25 (37.3)	20 (29.9)	67
1986	7	30 (33.3)	39 (43.3)	21 (23.3)	90
2002	12	12 (60.0)	6 (30.0)	2 (10.0)	20
2003	2	3 (60.0)	1 (20.0)	1 (20.0)	5
2004	13	53 (35.8)	72 (48.6)	23 (15.5)	148
Total	252	447 (29.4)	747 (49.2)	324 (21.3)	1518

^a Fifteen host plant species represented by ≤ 2 samples.

square = 40.06, df = 35, $p > 0.05$) but there was a significant trend toward higher frequency of the spotted morph in the 2000s than in the 1970s and 1980s. Proportions of the spotted morph increased significantly (GLM-qb, $p = 0.03$) whereas that of chequered (GLM-qb, $p = 0.42$) and dark forms (GLM-b, $p = 0.06$) decreased.

DISCUSSION

Uniform proportions of the melanic form of *A. bipunctata* at localities of the Czech Republic was reported previously (Honěk, 1975). The present study established the absence of significant variation among populations of different host plants and the absence of temporal variation in morph proportions. This result raises questions about the causes of such uniformity in central European populations

that is in contrast to the variation observed in populations of southern, western and northern Europe. *Adalia bipunctata* can be considered an example of a balanced polymorphism that is maintained by selection favouring the melanic morph during summer, balanced by greater mortality of this morph during winter (Lusis, 1961; Timofeeff-Ressovsky & Svirezhev, 1966; Brakefield, 1984b). However, the recent data of central European populations did not demonstrate seasonal changes. An earlier study using data from an overwintering site (Honěk, 1975) yielded a similar negative result. In the present study, using data from a light trap, the differences in frequency of melanics only approached the limit of significance. The differences might become significant if the numbers of coccinellids were greater, but the range of variation was low (maximum 20%). We suppose that temporal variation is limited by factor(s) that keep the proportion of melanics generally low. Seasonal variation may become manifest only in populations with a high overall proportion of melanics.

The mechanism involved in maintaining consistently low proportions of the melanic form in central European populations remains to be established. In central Europe, populations with $> 50\%$ melanic morph were common before the 1940s (Schröder, 1901, 1902; Meissner, 1907a,b; Dobzhansky, 1924;) and probably only disappeared between the 1940s and the 1970s. This is reflected in the change in proportion of melanics at Potsdam (Germany) where an original study of Timofeeff-Ressovsky established 25–75% of the melanic form in the 1930s (Timofeeff-Ressovsky, 1940; Timofeeff-Ressovsky & Svirezhev, 1966). Recently, the proportion of melanics was established at Geltow, c. 2 km distant from Potsdam. It had decreased to 15.2% ($n = 115$) in 1973 (Creed, 1975), and 5.0% ($n = 60$) in 1981 (A. Honěk, unpubl.). Thus, the potential for seasonal variation might have been eliminated by the overall decrease in proportion of melanic form in this area. Long-term changes in the proportion of the melanic form have been well documented in Great Britain (Brakefield & Lees, 1987) and Sankt Petersburg (Zakharov & Sergievsky, 1978) and reveal that melanics have decreased (UK) or increased (Russia) in parallel with changing industrial pollution. The present study does not indicate any correspondence between proportions of the melanic form and air pollution. Pollution in the Czech republic was maximal in the 1970s (no official data). Thereafter, emissions of solid particles (from major sources of pollution) decreased, from $971 \cdot 10^6$ kg in 1980, to $540 \cdot 10^6$ kg in 1988 and $15 \cdot 10^6$ kg in 2002 (Anonymous, 1990, 2004). This was not accompanied by any change in melanic frequency. Similar absence of change in the proportion of melanics despite increasing smoke pollution was also observed in a 50-year study of urban populations in Moscow (Zakharov, 1992). Both Russian and Czech studies were marked by a low proportion of melanic forms (mostly $< 10\%$), in contrast with the high proportion of melanics (20–80%) in Great Britain, Sankt Petersburg and The Netherlands. The significant long-term changes in morph proportions thus appeared

only under conditions that permitted large variation in the proportion of melanics. Abundant sunshine during the breeding period was hypothesized to be the main factor promoting low melanic frequency in central Europe (Honěk, 1975). Whereas climate change may favour a low frequency of melanics (de Jong & Brakefield, 1998), changes in frequencies during the early and late 20th century require further explanation.

Few available studies of *A. decempunctata* variation in western Europe indicated that morph proportions varied between local populations, but were rather similar over large geographic areas. In Great Britain, proportions of spotted, chequered and dark forms in central England were observed to be 53.1%, 33.5% and 13.4% (Brakefield, 1985) and 60.9%, 27.8% and 11.3% (Brakefield & Lees, 1987). Majerus (1994) found a similar range of morph proportions in 97 populations across the whole of Britain and did not observe any geographic trend in morph proportions. Proportions of the spotted, chequered and dark form were 32.7%, 42.1% and 25.2%, respectively in a sample of 2273 individuals from the Netherlands (Brakefield, 1985), also without a geographic trend in morph proportions, and are very similar to proportions found in the Czech Republic. Brakefield (1985) also did not find any difference between populations collected from different host plants. However, in all areas (Great Britain, The Netherlands, Czech Republic) variation in proportions of morphs was large, within the range of ca. 20% for each morph. This variation is much greater than for *A. bipunctata* and indicates the existence of particular factors that may influence the composition of local samples. One of these factors may be behavioural selection of microclimate which appears to vary among morphs (Stork et al., 2001).

Our data indicated a significant increase in proportion of the spotted form, the lightest of the three *A. decempunctata* morphs, over the 2000s. This was accompanied by a corresponding decrease in the relative frequency of chequered and dark forms. This increase in frequency of the non-melanic form might parallel the change observed in *A. bipunctata* populations of the Netherlands (de Jong & Brakefield, 1998) that was supposedly caused by climate change. However, further sampling should be conducted to confirm the observed trend because it is only evident in the few samples collected after 2000 when *A. decempunctata* was generally scarce. The large number of samples collected before 1990 when *A. decempunctata* was abundant did not indicate a long-term trend of increasing frequency of the spotted form.

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