Maintenance of colour polymorphism in the leaf beetle *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae: Paropsini)

HELEN F. NAHRUNG¹, & GEOFF R. ALLEN²

¹School of Natural Resource Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Queensland 4001, Australia. e-mail h.nahrung@qut.edu.au, and ²School of Agricultural Science, University of Tasmania, GPO Box 252-54, Hobart, Tasmania 7001, Australia (Accepted 10 October 2002)

Abstract

Intra-specific colour differences in insects may occur as a result of environmental factors such as food type, temperature and humidity, or may be under genetic control. These colour polymorphisms may result in fitness differences through several mechanisms, including mate selection, camouflage from or warning to natural enemies, and heat absorption. Two colour morphs of adult *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae: Paropsini) are present in the field in mainland Australia and Tasmania: a common green-brown form, and a rare black form. Tasmanian populations were sampled to assess the frequency of each morph at eight localities. The black form represented less than 3% of beetles (N=1724), with the proportion not significantly different between localities. Crossing tests using the two colour morphs of *C. agricola* showed that the black form was genetically dominant over the common form. To assess whether colour morphs had any fitness differences, we measured pre-oviposition period, fecundity, longevity, adult size and egg hatch rate, which each showed no significant difference between colour morphs. Field sampling of mating pairs and rearing the offspring of field-collected females showed no evidence of non-random mating. Modelling the population over 100 generations confirmed that for this species, melanism is controlled by a dominant but neutral allele, and, thus, is maintained at a constant low level in the population.

Keywords: *Melanism, neutral allele, Hardy-Weinberg*

Introduction

Colour polymorphism occurs in many insect species and may result in fitness differences through several mechanisms, including mate selection (e.g. Ueno et al. 1998), camouflage from natural enemies, aposematism (warning coloration) and heat absorption (e.g. Ottenheim et al. 1999; Majerus and Zakharov 2000). Intra-specific colour polymorphism may occur as a result of environmental factors such as food type, temperature and humidity, or it may be under genetic control (Verdyck et al. 1996). Colour polymorphism in insects occurs commonly through melanism, which is a trait for dark brown or black pigmentation in the cuticle. For example, syrphid flies vary in the degree of melanic banding depending on developmental temperature (Marriott and Holloway 1998) and in
the Mantodea, melanism can arise as a result of fire (Balderson 1991). Where coloration is under genetic control, natural selection eliminates unfavourable alleles from populations. Mechanisms leading to genetic polymorphisms have received much theoretical attention; for example, melanism in the peppered moth, *Biston betularia* L., increased by industrial pollution is considered a landmark case of natural selection (Majerus 1998).

Some species of paropsine chrysomelid beetles exhibit phenotypic dimorphism via melanism as adults. Different melanistic morphs of *Chrysophtharta cloelia* (Stål), *C. decolorata* (Chapuis) and *C. obovata* (Chapuis) are present in natural populations (Selman 1994). Black morphs of *C. variicollis* (Chapuis), *C. agricola* (Chapuis), *C. atlanta* (Blackburn), *Peltoschema hamadryas* (Stål), *P. oceanica* (Boisduval) and *P. rubiginosa* (Chapuis) are found in mainland Australia (C. A. M. Reid, Australian Museum, personal communication). Selman (1994) suggested that melanic forms in the Paropsini may arise from development under moist conditions. The elytra of the predominant morph of *C. agricola* are golden-brown in mainland Australia (Selman 1994), and are grey or red-brown with slight gold tessellation in Tasmania (de Little 1979). The black morph is entirely black, and it was this morph from which the original species description was made in 1877 in the genus *Paropsis* Olivier (de Little 1979). We assessed the frequency of the black morph in Tasmanian populations between July 1999 and April 2001.

We also investigated the inheritance and incidence of melanism in *C. agricola*. We measured the reproductive success of both colour morphs in the laboratory, and assessed beetles in the field to determine whether there was evidence for non-random mating between morphs. We further examined the genetics and evolution of this phenotypic dimorphism using simulation of populations over several generations.

**Materials and methods**

*Morph frequencies in the field*

Between July 1999 and April 2001, collections of adult *C. agricola* were made from eight sites up to approximately 235 km apart throughout Tasmania: Frankford, the Florentine Valley, Parkham, Blue Gum Knob, Scottsdale, Geeveston, Ellendale and Ridgley (Figure 1). The sex of field-collected black beetles was checked based on tarsal differences (Baly 1862), and frequencies of the black morph within each sex were scored and we tested whether these were significantly different. *C. agricola* specimens collected from mainland Australia and held in the Australian National Insect Collection (ANIC) were counted, and the number of black and normal morphs was recorded.

*Crossing experiments in the laboratory*

Nahrung and Reid (2002) found that less than 30% of beetles stored sperm during overwintering, so virgin females were collected as overwintering adults from the leaf litter of a *Eucalyptus globulus* plantation near Parkham, Tasmania. Sampling of approximately 1370 litres of leaf litter found 210 beetles of which just five were black. Upon collection, females were held individually in the laboratory until they began to produce eggs and if no eggs hatched after 2 weeks, females were deemed virgin and paired with males. Three black virgin females were identified. The following crosses comprised the parental (P) generation: black ♀–black ♂ (N=2); black ♀–normal ♀/black ♂–normal ♀ (N=2); normal ♀–normal ♂ (N=2).
The eggs from each parental cross were kept in petri dishes held at 21 ± 3°C, 16L:8D photoperiod and larvae were reared through to adulthood. There was no visible difference in eggs, larvae or pupae between colour morphs. Indeed, even newly emerged adults of each morph were indistinguishable from each other, as noted by de Little (1979). Teneral adults were held in petri dishes and plastic containers until they matured and could be scored for colour. Containers were kept in a controlled temperature room at 21 ± 3°C, 16L:8D photoperiod to prevent F1 adults entering diapause. Crosses between offspring of
the parental crosses were conducted by pairing males and females that had been held separately since pupation to guarantee virginity of females to produce F2 offspring.

Field mating behaviour of colour morphs

To determine whether there was evidence for non-random mating between morphs in the field, and to investigate whether field-collected black beetles were heterozygous or homozygous for the black allele, we reared the offspring of beetles that had mated in the field. Eighteen reproductively mature black females, two normal females collected whilst mating with black males and three reproductively mature normal females were collected between November and December 2000 from *Eucalyptus nitens* plantations at Frankford and the Florentine Valley. Adults and pairs were returned to the laboratory and held in separate petri dishes at the same temperature and photoperiod in which the crossing tests were conducted. Eggs produced were reared to adulthood to ascertain the ratio of black: normal offspring produced by field-matings.

Reproductive fitness

A total of 135 teneral, laboratory-reared beetles originating from the Florentine Valley were held in the laboratory (21 ± 3°C, 16L:8D) until they matured to obtain black or normal coloration, and had mated. Fourteen black adults (seven males, seven females) and 121 normal adults (81 males, 40 females) were transferred to separate petri dishes with moist filter paper, and provided fresh *E. nitens* foliage. Filter paper and foliage were changed twice each week, the number of eggs laid by females was counted, and their hatch rate was determined by counting the number of larvae that emerged. Adults were kept until they died to assess longevity.

To determine when each colour morph began oviposition following overwintering, normal females (N=26) and black females (N=2) were held at 9°C, and normal females (N=3) and black females (N=2) were held at 21°C after collection from overwintering sites. Pre-oviposition period was defined as the period between termination of diapause (feeding and frass production) and the beginning of egg production. The maximum body length of field-collected beetles was measured (±0.1 mm) using a digital caliper to determine whether either colour morph was significantly larger than the other.

Testing Hardy-Weinberg equilibrium, and population modelling

The populations collected from the field were assessed for Hardy-Weinberg equilibrium by determining the number and frequency of black (B=p) and normal (b=q) alleles, and comparing the field data with expected values p² (BB), 2pq (Bb) and q² (bb). We assumed that all field-collected black beetles were heterozygotes (Bb), consistent with the results of our field collections (N=23) (see below). Deviations between observed and expected morph frequencies were tested using a Chi-square goodness of fit.

We plotted a model of 100 successive generations of beetles, using a starting population of 100 individuals, with 3% heterozygous black (where B is black, dominant and b is normal, recessive).

Black adults were designated genotype Bb because the low number of black morphs in a population would mean that the probability of homozygous black beetles was extremely
low. Furthermore, the offspring ratios of black beetles collected in the field confirmed that all were heterozygotes \((N=23)\). No selective advantage for either morph, or assortative mating was included in the initial model because we observed neither in the laboratory or field (see results). We assumed a 1:1 sex ratio, and that each beetle mated only once. A 1:1 sex ratio is observed in the laboratory and field, but beetles of both sexes mate more than once under laboratory and field conditions (authors’ unpublished data). This does not affect the output of the model unless we assign differential mating success between matings over time, which we do not have data to quantify. The number of offspring surviving from each pair in each generation was given a random value between 0.1 and 5.0 to simulate varying survival in a fluctuating environment. To confirm that neither phenotype has a selective advantage over the other, the model was re-run allowing a 5% offspring survival advantage to black beetles. Further, a simulation incorporating a homozygous (BB) advantage of 20% was run, as was a model that was modified to demonstrate the effects of non-random mating, or differential sperm utilization patterns.

**Results**

Over 1700 beetles were collected from eight sites in Tasmania (Table I). Only sites at which more than 100 beetles were collected are shown separately. There was an overall frequency of 2.6% of black beetles and no significant difference in the frequency of black morph beetles between sites (two-way contingency table, \(\chi^2=1.77, P=0.62\)). There was also no difference between the overall number of black males and females collected \((\chi^2=0.07, P=0.79)\).

F1 and F2 laboratory crosses and field-collected offspring ratios indicated that black coloration was due to a dominant allele, with black and normal morphs produced from each combination except for normal–normal crosses (Table II). Hence, the normal coloration was found only in individuals that were homozygous for the recessive allele. Uniformly very dark brown adults were also produced by crosses involving black parents. One such specimen was found in the ANIC collection of *C. agricola*, and such beetles have been observed in the field (H. F. N., personal observation). Because they were more similar to the black form than to the normal form, we included them as black.

There was no evidence of assortative mating in the field, with all field-collected black females mating with normal males and producing normal and black offspring in an

| Site               | Percentage black phenotype (total N) |
|--------------------|--------------------------------------|
| Florentine Valley  | 2 (700)                              |
| Frankford          | 3.1 (503)                            |
| Parkham            | 2.4 (207)                            |
| Blue Gum Knob      | 2.2 (182)                            |
| Overall (Tasmania) | 2.6 (1724)                           |
| ANIC (mainland)    | 0.9 (519)                            |

‘Overall’ includes these sites, and four others at which black beetles were collected. The final row shows the percentage of black *C. agricola* specimens held in the Australian National Insect Collection (ANIC) collected from mainland Australia.
Table II. The offspring and expected ratios produced from laboratory crosses of black (B) and normal (N) adults, and of crosses between their offspring (F2s), and of field-collected, field-mated black (B) and normal (N) females, where X represents males of unknown phenotype.

| Cross (♀♂) | Offspring (B:N) | Expected ratio\(^{1}\) | Goodness of fit\(^{2}\) to expected ratio\(^{1}\) |
|------------|-----------------|------------------------|-----------------------------------------------|
| Parents    |                 |                        |                                               |
| BB         | 49:17           | 3:1                    | \(\chi^2=0, \ P>0.99\)                        |
| BN         | 17:17           | 1:1                    |                                               |
| NB         | 23:20           | 1:1                    | \(\chi^2=0.92, \ P=0.66\)                    |
| NN         | 0:38            | 0:1                    |                                               |
| F2s        |                 |                        |                                               |
| Parents BB |                 |                        |                                               |
| NB         | 2:0             | 1:0 or 1:0             | \(\chi^2=0.67, \ P=0.4\)                    |
| NB         | 9:6             | 1:1 or 1:0             |                                               |
| NB         | 16:8            | 1:0 or 3:1             | \(\chi^2=2.7, \ P=0.1\)                    |
| BB         | 7:0             | 1:0 or 3:1             |                                               |
| BB         | 2:0             | 1:0 or 3:1             |                                               |
| BB         | 3:0             | 1:0 or 3:1             |                                               |
| BB         | 12:4            | 1:0 or 3:1             |                                               |
| BB         | 5:0             | 1:0 or 3:1             |                                               |
| BB         | 4:0             | 1:0 or 3:1             |                                               |
| NN         | 0:7             | 0:1                    |                                               |
| NN         | 0:9             | 0:1                    |                                               |
| Parents BN/NB |            |                        |                                               |
| NB         | 8:13            | 1:1                    | \(\chi^2=0.76, \ P=0.62\)                    |
| BB         | 8:4             | 1:0 or 3:1             | \(\chi^2=0.11, \ P=0.74\)                    |
| BB         | 4:4             | 1:0 or 3:1             | \(\chi^2=1.5, \ P=0.22\)                    |
| BB         | 3:0             | 1:0 or 3:1             |                                               |
| Parents NN |                 |                        |                                               |
| NN         | 0:14            | 0:1                    |                                               |
| Field-collected |            |                        |                                               |
| BX1        | 6:8             | 1:1                    | \(\chi^2=0.36, \ P=0.55\)                    |
| BX2        | 17:11           | 1:1                    | \(\chi^2=1.32, \ P=0.75\)                    |
| BX3        | 13:6            | 1:1                    | \(\chi^2=2.63, \ P=0.1\)                     |
| BX4        | 20:28           | 1:1                    | \(\chi^2=1.35, \ P=0.25\)                    |
| BX5        | 15:14           | 1:1                    | \(\chi^2=0.07, \ P=0.79\)                    |
| BX6        | 7:12            | 1:1                    | \(\chi^2=1.37, \ P=0.76\)                    |
| BX7        | 17:19           | 1:1                    | \(\chi^2=0.14, \ P=0.7\)                     |
| BX8        | 33:32           | 1:1                    | \(\chi^2=0.03, \ P=0.9\)                     |
| BX9        | 28:28           | 1:1                    |                                               |
| BX10       | 10:4            | 1:1                    | \(\chi^2=2.6, \ P=0.1\)                      |
| BX11       | 11:13           | 1:1                    | \(\chi^2=0.21, \ P=0.7\)                     |
| BX12       | 19:16           | 1:1                    | \(\chi^2=0.29, \ P=0.6\)                     |
| BX13       | 11:6            | 1:1                    | \(\chi^2=1.1, \ P=0.3\)                      |
| BX14       | 8:10            | 1:1                    | \(\chi^2=0.3, \ P=0.6\)                      |
| BX15       | 10:9            | 1:1                    | \(\chi^2=0.1, \ P=0.8\)                      |
| BX16       | 11:8            | 1:1                    | \(\chi^2=0.5, \ P=0.5\)                      |
| BX17       | 12:12           | 1:1                    |                                               |
| BX18       | 17:23           | 1:1                    | \(\chi^2=0.9, \ P=0.3\)                      |
| Total BX   | 265:259         | 1:1                    |                                               |
| NB1        | 21:22           | 1:1                    | \(\chi^2=0.05, \ P=0.82\)                    |
| NB2        | 32:30           | 1:1                    | \(\chi^2=0.08, \ P=0.78\)                    |
| Total NB   | 53:52           | 1:1                    |                                               |
| NX19       | 0:6             | 0:1                    |                                               |
| NX20       | 0:3             | 0:1                    |                                               |
| NX21       | 0:9             | 0:1                    |                                               |
| Total NX   | 0:18            | 0:1                    |                                               |
expected 1:1 ratio (Table II). These results therefore infer that all field males denoted X1–X21 in Table II were normal. Furthermore, identical ratios were obtained using females that had mated in the field as with females that mated with normal males in the laboratory: black females produced black and normal offspring in a 1:1 ratio, and normal females produced only normal offspring. Hence, all field-collected black beetles of both sexes were heterozygotes, and all normal beetles were homozygous for the recessive allele.

We found no differences between black and normal beetles for the female reproductive fitness parameters that we measured in the laboratory (Table III). Furthermore, there was no significant difference in adult longevity or size of colour morphs for either sex.

The populations collected from the field were found to be in Hardy-Weinberg equilibrium (Table IV).

In the neutral model, the proportion of black individuals remained constant (3%) in the population (Figure 2), suggesting that the population is at Hardy-Weinberg equilibrium and that black is a dominant but neutral allele. Re-running the model giving a 5% offspring survival advantage to black morphs demonstrated that if either form were selectively favoured, the other would eventually become extinct. Similarly, if there were a homozygous advantage to black individuals (BB), the proportion of black beetles would increase. If there were any assortative mating or sperm utilization strategies so that only similar phenotypes reproduced with each other, black beetles would become very rare.

Table III. Comparison between black and normal beetles for fitness parameters measured in the laboratory and between size of field-collected beetles.

| Parameter                                           | Black beetles | Normal beetles |
|-----------------------------------------------------|---------------|----------------|
| Mean ± SE pre-oviposition period at 9°C (days)      | 37 ± 13       | 42.6 ± 21      |
|                                                     | (N=2)         | (N=3)          |
| Mean ± SE pre-oviposition period at 21°C (days)     | 10.5 ± 2.1    | 7.8 ± 0.7      |
|                                                     | (N=2)         | (N=26)         |
| Mean ± SE fecundity (eggs per day)                  | 6.4 ± 1.2     | 7.6 ± 0.5      |
|                                                     | (N=7)         | (N=40)         |
| Overall egg hatch rate (%)                          | 78.4          | 75.1           |
|                                                     | (N=7)         | (N=40)         |
| Mean ± SE adult longevity (days)                    | 187.6 ± 32.9  | 213.3 ± 8      |
|                                                     | (N=14)        | (N=121)        |
| Mean ± SE male length (mm)                          | 8.4 ± 0.1     | 8.4 ± 0.08     |
|                                                     | (N=12)        | (N=50)         |
| Mean ± SE female length (mm)                        | 9.1 ± 0.1     | 8.9 ± 0.1      |
|                                                     | (N=19)        | (N=50)         |

No significant differences were recorded between morphs for any parameter (t-test, P>0.05). The length of female beetles was significantly greater than that of males (t-test, t_{120}=4.37, P<0.01).
Discussion

Here we have demonstrated that the black morph present in low levels in field populations of *Chrysophtharta agricola* is under genetic control and not by development under humid conditions as suggested by Selman (1994) for other beetles in the Paropsini. In *C. agricola*, melanism is controlled by a dominant, neutral allele that is maintained under

| Site          | Allele frequency | Allele frequency | BB   | Bb   | bb   |
|---------------|------------------|-------------------|------|------|------|
| Florentine    | 0.01             | 0.99              | 0    | 14   | 686  |
| Valley        |                  | Expected          | 0.07 | 13.9 | 686.1|
| Frankford     | 0.016            | 0.984             | 0    | 16   | 487  |
|               |                  | Expected          | 0.13 | 15.8 | 487  |
| Parkham       | 0.012            | 0.99              | 0    | 5    | 202  |
|               |                  | Expected          | 0.03 | 4.9  | 202.9|
| Blue Gum      | 0.011            | 0.99              | 0    | 4    | 178  |
| Knob          |                  | Expected          | 0.02 | 3.9  | 178  |
| Overall       | 0.013            | 0.987             | 0    | 44   | 1680 |
|               |                  | Expected          | 0.3  | 44.2 | 1679.5|

For each site, the observed and expected frequencies did not differ (Chi-square goodness of fit, $P>0.9$). Where B is black, dominant and b is normal, recessive.

Figure 2. Simulation of 100 successive generations of a *Chrysophtharta agricola* population, showing the proportion of black morphs and log (population size) for different selection assumptions. Black morphs were initially represented by 3% of the population, and were heterozygous (Bb), allowing: (i) no selective advantage to either morph; (ii) black morphs with 5% offspring survival advantage over normal morphs; (iii) homozygous black morphs (BB) with 20% offspring survival advantage over all other genotypes; (iv) black adults mated only with other black adults, and normal adults mated only with normal adults.
Hardy-Weinberg equilibrium. Collection records suggest that the melanic morph of *C. agricola* has been present at a low frequency in *C. agricola* populations for at least the last 120 years. The black allele is not eliminated by natural selection because it appears neither favourable nor unfavourable to survival or reproduction. Furthermore, the black allele has persisted in both mainland Australia and Tasmania despite the separation of the two land masses over 10 000 years ago forming a likely vicariance barrier (Cranston and Naumann 1991).

Under laboratory conditions we found no differences in reproductive fitness between black and normal beetles. In other organisms, melanism is associated with thermal advantages through increased heat absorption, and in the two-spot ladybird, *Adalia bipunctata*, this was postulated as the principal factor influencing differences in morph activity (de Jong et al. 1996). Like *C. agricola*, *A. bipunctata* is polymorphic for colour and under genetic control in which the non-melanic form is homozygous recessive. However, unlike *C. agricola*, *A. bipunctata* shows clinal variation in the frequency with which melanic and non-melanic morphs occur, coinciding with an increase in temperature (de Jong and Brakefield 1998). Although our experiments were restricted to artificial light and we did not test fitness differences under solar radiation we have not observed the activity of black beetles in the field to differ from that of normal beetles.

We found that there was no assortative mating between the two colour morphs of *C. agricola*. Fujiyama and Arimoto (1988) likewise found random mating and Mendelian inheritance between the two colour forms of another species of chrysomelid beetle, *Chrysolina aurichalcea* (Mannerheim). Selman (1994) reported that adult paropsine beetles are largely immune to predation by birds and mammals, so it is unlikely that either morph of *C. agricola* is selectively disadvantaged through conspicuousness to predators.

The invasion and persistence of the rare neutral allele causing melanism into *C. agricola* populations may have occurred through several means, including past selection pressure which favoured the black allele, a genetic bottleneck, or because the black allele appeared during a rapid and long-lasting population increase. Indeed, the long-term probability of persistence of a newly arisen mutant is greater than zero if the rate of increase of this genotype is greater than one (Haldane 1930). Even if the rate of increase is less than or equal to one (but >0.5), the probability of an unfavourable mutant persisting in the population is greater than zero as long as the population size is increasing (Ewens 1967). Hence, a neutral allele can invade a population. Once the number of the neutral alleles is large enough it will not be affected by genetic drift and will attain Hardy-Weinberg equilibrium. However, slight selection pressure against low-frequency alleles may be counteracted by mutation pressure, although we have no evidence to suggest that this is the mechanism behind maintenance of the black allele in *C. agricola* populations.

The mechanism by which the black form came to represent 3% of the population is unknown and we propose three hypotheses to explain this, assuming that the mutation happened only once. The black mutation may have occurred in one individual in a small population; thus, the single mutation represented 3% of the population upon its appearance, and, being neither advantageous or deleterious, remained at that level as the population expanded and dispersed. Indeed, since the black morph is so geographically widespread we assume that the mutation arose more than 10 000 years ago before populations became isolated by the separation of Tasmania from mainland Australia. Alternatively, at the time of its appearance, or at some time in the past, the black morph may have held a selective advantage over the normal morph and was selected over a number of generations to attain 3% of the population before the advantage was lost.
Finally, the proportion of black morphs may have reached 3% because it may have appeared during a time of rapid population expansion before a genetic bottleneck that favoured the black morph.

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Appendix 1

Calculations used for modelling C. agricola populations (Figure 1)

For starting population where normal adults are genotype bb and black adults are genotype Bb:

Number of normal adults in population at generation $t=a(t)$
Number of black adults in population at generation $t=b(t)$
Normal $\zeta=a/2=NM(t)$
Normal $\varphi=a/2=NF(t)$
Black $\zeta=b/2=BM(t)$
Black $\varphi=b/2=BF(t)$.

Probability of mating for each mating combination:

Black $\varphi \times$ Normal $\zeta = \frac{(BF(t) \times NM(t))}{(NM(t)+BM(t))}=c(t)$
Black $\varphi \times$ Black $\zeta = \frac{(BF(t) \times BM(t))}{(NM(t)+BM(t))}=d(t)$
Normal $\varphi \times$ Black $\zeta = \frac{(NF(t) \times BM(t))}{(NM(t)+BM(t))}=e(t)$
Normal $\varphi \times$ Normal $\zeta = \frac{(NF(t) \times NM(t))}{(NM(t)+BM(t))}=f(t)$.

Offspring produced from each mating combination $= y(t)$ (random between 0.1 and 5), so

Black $\varphi \times$ Normal $\zeta = c(t)y(t)$
Black $\varphi \times$ Black $\zeta = d(t)y(t)$
Normal $\varphi \times$ Black $\zeta = e(t)y(t)$
Normal $\varphi \times$ Normal $\zeta = f(t)y(t)$.

Offspring types from each mating combination using Mendelian inheritance ratios:

Black $\varphi \times$ Normal $\zeta$: black offspring $= c(t)y(t)/2$, normal offspring $= c(t)y(t)/2$
Black $\varphi \times$ Black $\zeta$: black offspring $= d(t)y(t) \times 0.75$, normal offspring $= d(t)y(t) \times 0.25$.
Normal $\varphi \times$ Black $\delta$: black offspring $= e(t)y(t)/2$, normal offspring $= e(t)y(f)/2$

Normal $\varphi \times$ Normal $\delta$: normal offspring $= f(t)y(t)$.

Thus, generation $(t+1)$ has $[(c(t)y(t)/2) + (d(t)y(t) \times 0.75) + (e(t)y(t)/2)]$ black adults, and $[(c(t)y(t)/2) + (d(t)y(t) \times 0.25) + (e(t)y(t)/2) + (f(t)y(t))$ normal adults.

The number of adults of each genotype for this generation is calculated by:

$$
BB = (d(t)y(t) \times 0.75) \times 0.333
$$

$$
Bb = [d(t)y(t) \times 0.75] + [e(t)y(t)/2] + [e(t)y(t)/2]
$$

$$
bb = (d(t)y(t) \times 0.25) + (e(t)y(t)/2) + (e(t)y(t)/2) + (f(t)y(t))
$$

and the number of each gender for each genotype is calculated by:

$$
BB \varphi = BB/2 = g
$$

$$
BB \delta = BB/2 = h
$$

$$
Bb \varphi = Bb/2 = i
$$

$$
Bb \delta = Bb/2 = j
$$

$$
bb \varphi = bb/2 = k
$$

$$
bb \delta = bb/2 = l
$$

From these, the number of matings of each combination is calculated thus:

$$
Bb \varphi \times bb \delta = il/(h+j+l) = m
$$

$$
BB \varphi \times bb \delta = gl/(h+j+l) = n
$$

$$
BB \varphi \times Bb \delta = gi/(h+j+l) = o
$$

$$
BB \varphi \times BB \delta = gh/(h+j+l) = p
$$

$$
bb \varphi \times Bb \delta = kj/(h+j+l) = q
$$

$$
bb \varphi \times BB \delta = kh/(h+j+l) = r
$$

$$
bb \varphi \times bb \delta = kl/(h+j+l) = s
$$

$$
Bb \varphi \times Bb \delta = ij/(h+j+l) = t
$$

$$
Bb \varphi \times BB \delta = ih/(h+j+l) = u.
$$

Each of these matings ($m$, $n$, $o$, $p$, $q$, $r$, $s$, $t$ and $u$) produces $y$ offspring, to which Mendelian inheritance ratios are applied thus:

$$
Bb \varphi \times bb \delta: \text{black offspring} = my/2, \text{normal offspring} = my/2
$$

$$
BB \varphi \times bb \delta: \text{black offspring} = ny
$$

$$
BB \varphi \times Bb \delta: \text{black offspring} = oy
$$

$$
BB \varphi \times BB \delta: \text{black offspring} = py
$$

$$
bb \varphi \times Bb \delta: \text{black offspring} = qy/2, \text{normal offspring} = qy/2
$$

$$
bb \varphi \times BB \delta: \text{black offspring} = ry
$$

$$
bb \varphi \times bb \delta: \text{normal offspring} = sy
$$

$$
Bb \varphi \times Bb \delta: \text{black offspring} = ty \times 0.75, \text{normal offspring} = ty \times 0.25
$$

$$
Bb \varphi \times BB \delta: \text{black offspring} = uy.
$$

From these, the number of black $[(my/2)+ny+oy+py+(qy/2)+ry+(ty \times 0.75)+uy]$ and normal $[(my/2)+(qy/2)+sy+(ty \times 0.25)]$ offspring are calculated, and the proportion of black adults is determined by: black/(black+normal), and the population size by (black+normal).
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