Hybrid effects in field populations of the African monarch butterfly, Danaus chrysippus (L.) (Lepidoptera: Nymphalidae)

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Received 9 January 2021; revised 13 February 2021; accepted for publication 13 February 2021

Heterosis, Haldane and Bateson-Dobzhansky-Muller effects have been widely documented amongst a range of plants and animals. However, typically these effects are shown by taking parents of known genotype into the laboratory and measuring components of the F1 progeny under laboratory conditions. This leaves in doubt the real significance of such effects in the field. Here we use the well-known colour pattern genotypes of the African monarch or queen (Danaus chrysippus), which also control wing length, to test these effects both in the laboratory and in a contact zone in the field. By measuring the wing lengths in animals of known colour pattern genotype we show clear evidence for all three hybrid effects at the A and BC colour patterning loci, and importantly, that these same effects persist in the same presumptive F1s when measured in hybrid populations in the field. This demonstrates the power of a system in which genotypes can be directly inferred in the field and highlights that all three hybrid effects can be seen in the East African contact zone of this fascinating butterfly.

ADDITIONAL KEYWORDS: Asymmetric crossing – Bateson-Dobzhansky-Muller effect – body size – climate change – Haldane rule effect – heterosis – migration – non-random mating – reticulate evolution – speciation – wing length.

INTRODUCTION

In crosses between genetically diverse parents, greater body size or fertility of an F1 progeny, such that it is either superior to the better of two parents (Mather & Jinks, 1982), or exceeds the mid-parent value (Falconer & Mackay, 1995), is known as heterosis. The phenomenon was first identified as hybrid vigour by both Darwin (1876) and Wallace (1889). Dobzhansky (1950, 1952) distinguished ‘euheterosis’ (in which Darwinian fitness, estimated as fecundity or longevity, is improved in F1 hybrids) from ‘luxuriance’, where the offspring are enhanced in a purely metric sense. ‘Euheterosis’ corresponds to ‘heterozygote advantage’ (Sheppard, 1967; Ford, 1971) or ‘overdominance’ (Wallace, 1970), whereas ‘luxuriance’ is synonymous with ‘hybrid vigour’ (Smith, 1980). As we have not estimated the fecundity or longevity of the F1, we are unable to distinguish ‘euheterosis’ from ‘luxuriance’; however, in crosses between wild, genetically deviant phenotypes, the latter is the likely model.

All genetic models for heterosis (Birchler et al., 2006; Oakley et al., 2015) hold that it is controlled...
by several loci, linked or unlinked, and attribute the heightened fitness of the F₁, compared to its parents, to the masking of deleterious recessive alleles. According to one heterosis model—associative overdominance (Ohta & Kimura, 1970)—the masking of lethal or semi-lethal alleles is secured by recombination suppression and linkage disequilibrium. However, in practice, determining the precise genetic basis for heterosis is difficult because of undetected (or unexplained) epistasis and the many unknown genes that may contribute to the effect.

Haldane (1922) stated ‘When in the F₁ offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous [heterogametic] one’. Haldane found that in animals with an XY mechanism for sex determination (e.g. mammals and most insects), the sex adversely affected in the F₁ was invariably the heterogametic (XY) male, whereas in ZW animals (e.g. birds and Lepidoptera) it was the female. Recognition of Haldane’s rule in dioecious plants such as Silene (Brothers & Delph, 2010), haplodiploid wasps (Koevoets & Beukeboom, 2009) and hermaphroditic pulmonates (Mollusca, Gastropoda) (Schilthuizen et al., 2011) have hoisted its applicability towards universality (Coyne & Orr, 1989, 2004; Laurie, 1997); the Haldane rule ‘represents a nearly obligatory first step in the evolution of postzygotic isolation’ and, therefore, of speciation (Coyne & Orr, 1989). For a ZW butterfly, the cross Danaus [chrysippus] klugii (male) × Danaus gilippus berenice (female), which Ackery & Vane-Wright (1984) had suggested might be conspecific, is illustrative. The ZZ F₁, males were viable whereas the ZW females, though equal in number, were inviable (Smith et al., 2002); thus, the two are indeed distinct species.

Several genetic models have attempted to interpret the Haldane rule in diploid organisms (Charlesworth et al., 1987; Hurst & Pomiankowski, 1991; Turelli & Orr, 2000; Tao & Hartle, 2003; Wang, 2003; Delph & Demuth, 2016); however, the dominance hypothesis (Haldane, 1932; Muller, 1942) is the most generally applicable. The dominance hypothesis postulates that heterogametic hybrids—the ZW female in all Lepidoptera (Sturtevant, 1915; Suomalainen et al., 1973; Prowell, 1998; Traut et al., 2008)—are affected by all Z-linked alleles, whether dominant or recessive, thus causing incompatibility when divergent alleles are brought together. Whereas ZZ (male) hybrids are only affected by dominant deleterious alleles, ZW (female) hybrids, which carry only one copy of any Z-linked gene, will be affected by all deleterious mutations regardless of dominance. As a result, hybrid inferiority is more evident in ZW females than ZZ males in butterflies (Turelli & Orr, 1995).

In large, outbred and more anciently-diverged populations, Bateson-Dobzhansky-Muller (BDM) effects (Bateson, 1909; Dobzhansky, 1933, 1936; Muller, 1939), otherwise known as ‘outbreeding depression’ or ‘underdominance’, is caused by genome-wide autosomal incompatibilities and equally affects both sexes (Wallace, 1889; Lynch, 1991; Orr & Turelli, 2001; Orr, 2005; Kirkpatrick & Barton, 2006). BDM is a model for an isolation mechanism which applies when both sexes of the F₁ have lower fitness than the parents, whereas the Haldane rule is a trend which is observed only in the heterogametic sex.

The African monarch or queen butterfly, Danaus chrysippus (Linnaeus, 1758), comprises several named taxa which are largely geographically separated but cohabit and reticulate on a seasonal or semi-permanent basis through a large area of East-Central Africa. In previous papers, we have designated the area of hybridism as the contact zone (Smith et al., 2016: fig. 1). At first description most of the geographical colour forms of D. chrysippus were described by their protologue authors (Linnaeus, 1758; Schreber, 1759; Cramer, 1777; Klug, 1845; Moore, 1883; Butler, 1886) as species. In more recent times, however, D. chrysippus populations in Africa have been split on the basis of characteristically predominant phenotypes (Smith et al., 2019: fig. 2) which have been variously designated by the many authors involved as forms, races, polymorphs, genotypes, subspecies or semispecies of D. chrysippus. Genomic analyses have revealed that these polymorphisms are largely panmictic across much of the genome, with the exception of a few strongly differentiated ‘islands of divergence’. These include a broad region of suppressed recombination on chromosome 15 (chr15) containing the B and C colour patterning loci (hereafter termed the ‘BC’ locus), and a similar region on chromosome 4 (chr4), putatively containing the A locus (Martin et al., 2020). The precise geographical distributions of these divergent alleles, and frequency clines between them, remain to be described using molecular analyses across the range of the species.

It is important at this stage to point out to all readers that two changes to the nomenclature of D. chrysippus phenotypes are adopted in this paper (Table 1). First, the phenotype long known as dorippus (Klug, 1845), genotype bC/bC (Table 1), is re-named klugii (Butler, 1886), as recommended by Vane-Wright (2020). Second, as the name klugii is no longer available (Smith et al., 2019: fig. 2) which have been variously designated by the many authors involved as forms, races, polymorphs, genotypes, subspecies or semispecies of D. chrysippus. Genomic analyses have revealed that these polymorphisms are largely panmictic across much of the genome, with the exception of a few strongly differentiated ‘islands of divergence’. These include a broad region of suppressed recombination on chromosome 15 (chr15) containing the B and C colour patterning loci (hereafter termed the ‘BC’ locus), and a similar region on chromosome 4 (chr4), putatively containing the A locus (Martin et al., 2020). The precise geographical distributions of these divergent alleles, and frequency clines between them, remain to be described using molecular analyses across the range of the species.

The D. chrysippus polymorphism in East Africa is contentious because the butterfly is distasteful and therefore all morphs are aposmatic. Although it does not exactly violate Ford’s commonly accepted definition of the term “genetic polymorphism is the occurrence together in the same locality of two or more discontinuous
forms of a species in such proportions that the rarest of them cannot be maintained by recurrent mutation” (Ford, 1940), it disobeys the spirit. Ford’s definition, arguably, was envisaged to apply principally to polymorphic animals, such as snails and camouflaged moths, which display phaneropolymorphism (Huxley, 1955), individual morphs being either cryptic or Batesian mimics which are female-limited with free interbreeding among morphs. Although *D. chrysippus* polymorphisms have a geographic basis (Ford,
Figure 2. Haldane rule effect at the BC locus (Supporting Information, Table S8) in wild-collected butterflies from the contact zone (data from DB4). The presumptive cross is orientis (Bc/Bc) × klugii (bC/bC). In the F₁, heterozygote (Bc/bC) females (A) are significantly smaller than both their homozygous parents, whereas among male F₁ genotypes (B) there are no significant size differences.
1940), they are seasonally variable and show a replicable pattern from year to year maintained by a migratory response to movements of the intertropical convergence zone (ITCZ) (Smith et al., 1997). Partial assortative mating among morphs has been observed in regions of polymorphism (Gordon, 1984; Smith, 1984). In the light of what we now understand, D. chrysippus polymorphism is better described by the term ‘admixture polymorphism’, a term possibly first used by Wall (2000). As an alternative to Fordian polymorphism, admixture polymorphism is a gathering together on an ephemeral basis of distinct populations which are insufficiently diverged to be considered species and which may interbreed on a restricted and fortuitous basis. Notwithstanding the foregoing, Fordian polymorphism, whether transient or stable, has probably evolved from admixture polymorphism in D. chrysippus. As differential predation among morphs has been deduced from beak marks on the wings (Smith, 1979) and recapture data (Gordon et al., 2010), polymorphic Müllerian mimicry, as in Heliconius numata (Jay et al., 2021), has possibly evolved in D. chrysippus in some instances where polymorphism exists throughout the year.

Complete dominance has not evolved at any of the A and BC loci alleles which control aspects of colour pattern (Smith et al., 1997; Martin et al., 2020; Table 1: Fig. 1), suggesting that most hybridisms among the homozygous forms are relatively recent (< 2 Mya BP) events (Fisher, 1930; Clarke & Sheppard, 1960; Sheppard, 1967). Although all D. chrysippus morphs are inter-fertile (Poulton, 1925; Owen & Chanter, 1968; Clarke et al., 1973; Smith, 1975; Gordon et al., 1984), they also display either assortative mate choice (Gordon, 1984; Smith, 1984) or, when enforced by sex ratio differences, disassortative mating (Smith, 1973; Gordon et al., 2014).

For wing length in butterflies is a convenient proxy for body size as it is easily measured in both field and laboratory, and is constant throughout adult life—in contrast to body weight which fluctuates on a daily basis in response to various activities (Edgar & Culvenor, 1974; Edgar et al., 1976; Boppré, 1983; Schulz et al., 1993). Therefore, we here use wing length as a proxy for fitness to investigate the basis of different hybrid effects in crosses between morphs controlled by the A and BC colour pattern loci. Specifically, in the light of the lack of field-derived data showing heterosis and/or Haldane effects in natural populations, we use these well-defined colour pattern genotypes (Table 1; Supporting Information, Fig. S2) to test for these effects both in the laboratory and, more importantly, in the field itself. As we have not directly estimated the fitness of the F1, we are unable to disentangle ‘euheterosis’ from ‘luxurience’, however, in crosses between wild genetically distinct phenotypes, the latter is more probable.

### MATERIAL AND METHODS

The data for this study span 45 years from 1974 to 2019: the sampling area is detailed in Supporting Information (Table S1). Four databases (DB1-4) comprise 4685 butterflies (2088 males and 2597 females), including all 18 colour genotypes (Table 1; Supporting Information, Table S1). Because the B and C loci are linked within a supergene, within which

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**Table 1.** The colour genotypes and phenotypes (numbers in bold) of D. chrysippus in Africa. The four morphs that are also considered as semi-species in the largely monomorphic parts of their range (outside the contact zone) are italicized whereas names of hybrid phenotypes are in Roman type enclosed within single quotes (Vane-Wright, 2020)

| A locus | BC locus |
|---------|----------|
| bc/bc   | Bc/Bc    |
| A/A 1   | 1.1 1.2  |
| chrysippus | orientis |
| A/A 2   | 2.1§ 2.2§ |
| ‘semialcippus’ | ‘semialcippus’ |
| a/a 3   | 3.1 3.2 |
| alcippus | alcippus |

| bC/bc  | bC/bc  | bC/bc  | bC/bc  | bC/bc  | bC/bc  |
|--------|--------|--------|--------|--------|--------|
| 1.3    | 1.4    | 1.4    | 1.5    | 1.6    |
| ‘transiens’ | ‘infumata’ | unnamed |
| 2.3    | 2.4    | 2.5    | 2.6§   |
| ‘semialbinus’ | ‘semialbinus’ | ‘semialbinus’ | ‘semialcippus’ |
| 3.3    | 3.4    | 3.5    | 3.6    |
| ‘albinus’ | ‘albinus’ | ‘albinus’ | alcippus |

Hybrids that occur in the contact zone alongside the four above-mentioned morphs are as follows: F1, hybrid form ‘transiens’ Suffert, 1900, from the cross 1.1 × 1.3; F1, hybrid f. ‘infumata’ Aurivillius, 1899, from the cross 1.2 × 1.3; unnamed F1, hybrid from cross 1.1 × 1.2; F1, hybrid f. ‘semialcippus’§, from crosses 1.1 × 3.1, 1.2 × 3.2 and 1.6 × 3.6; F1, hybrid f. ‘semialcippus’ Strand, 1910, from crosses 1.3 × 3.3, 1.4 × 3.4 and 1.5 × 3.5; F1, and backcross hybrid f. ‘albinus’ Lanz, 1896. The A locus on chr4 has two alleles, A (orange/brown hindwing) and a (white hindwing). The BC supergene on chr15 has three BC alleles: bc, as in D. chrysippus, 1.1 and D. alcippus, 3.1-3.2; Bc, as in D. orientis, 1.2; BC, as in D. klugii, 1.3. A fourth possible allele, BC, is vanishingly rare. §Introduction of the name ‘semialcippus’ for the African hybrid (Aa), formerly named ‘alcippoides’, is new. The name ‘alcippoides’ was given by Moore (1883) to an Asian form; as this form may have either the Aa or AA genotype, it is inappropriate for the African Aa form.

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RESULTS

Wing length in the wild-collected groups (DB1 + DB3) differ from the laboratory-reared groups (DB2 + DB4) by around 4 mm in both sexes ($P = 4.42 \times 10^{-6}$), presumably because laboratory-reared larvae are better nourished and protected. The wing length of males significantly exceeds that of females in both the laboratory-reared ($P < 0.001$) and wild-measured ($P < 0.001$) groups, as previously reported (Smith, 1980).

Seasonal variation in the wing lengths of males and females at Dar es Salaam from 1974–5 is shown in Supporting Information, Figure S2A. The beneficial effect of the main rains in April–May on size in the following months is clear. Although males are significantly larger than females in January–February and in July through to December, this size advantage at Dar es Salaam disappears in March–June. It is possible that the relative decline in male size in March–June is due to the arrival from the south of the Bc/c (orientis) genotype which is significantly smaller than the $bC/b-$ (klugii) genotype which it partially replaces (Supporting Information, Fig. S2C).

At the A locus (Supporting Information, Fig. S2B-C), frequency of the $Aa$ genotype, though always low at Dar es Salaam, increases in June-August, especially in males (Supporting Information, Fig. S2C).
the BC supergene locus (Supporting Information, Fig. S2D-E), bC/b- (klugii) is the most abundant in January-March while Bc/- (orientis) peaks in May-August. Whilst accepting that dispersal movements within a home range must be distinguished from genuine long-distance migration—in which movement to a new and distant home range is involved (Dingle, 2014)—these frequency changes (Supporting Information, Fig. S2) undoubtedly result from the latter (Smith & Owen, 1997; Smith et al., 1997; Lushai et al., 2003).

After controlling for seasonal changes in wing length, in laboratory-reared females at Dar es Salaam there are significant size differences between all genotypes at the A locus (Fig. 1; Supporting Information, Figs S3A, S5A; Table S2). The A- genotype (klugii and orientis) is larger than aa (alcippus) and the Aa genotype, being the largest, shows significant positive heterosis. In laboratory-reared males (Fig. 1; Supporting Information, Figs S3B, S5B; Table S2), the F₁ genotype Aa is significantly larger than both parental genotypes; however, in this case, there is no significant difference between A- and aa. In wild-caught females at Dar es Salaam (Supporting Information, Figs S3C, S5C; Table S3), there are no significant differences among genotypes; however, as 96% of the sample was A-, scarcity of Aa and aa genotypes affects this result. In wild-caught males (Supporting Information, Figs S3D, S5D; Table S3), as Aa is significantly larger than A-, there is again evidence for positive heterosis in males. Supporting Information, Figure S3E, shows frequency distributions for forewing length in wild-caught females from data sets throughout the contact zone (DB4), with control for collecting site but not for season. Frequency distributions for forewing length in males (Supporting Information, Figs S3F, S5F; Table S4), with control for collecting sites, once more shows that the Aa class is larger than both parents but significantly so only compared to the A- parental class.

In summary, there is strong evidence for positive heterosis at the A locus in males, both at Dar es Salaam and in the wider contact zone. For females, evidence for heterosis that is statistically significant is confined to the laboratory-bred sample; however, the Aa and aa genotypes were inadequately represented in the field samples (Supporting Information, Tables S3-S4). Hence, we conclude that there is evidence for A locus heterosis in both sexes. The laboratory-reared broods suggest that the A- class is larger than the aa class. For both sexes, the A locus results are conservative since penetrance of the a allele in Aa heterozygotes is 62.5% (N = 56) in males and only 24.5% (N = 51) in females (Smith, 1998); thus, on average, only 43.5% of butterflies could be genotyped visually for the A locus. The interaction for penetrance at the A locus vs. sex is highly significant, $\chi^2_{1} = 14.78$, $P = 0.0001$; however, there are no significant size differences or interactions with the BC loci (Smith, 1998: table 6).

In laboratory-reared females at Dar es Salaam (DB1, Fig. 2; Supporting Information, Figs S4A, S5G; Table S5) there are significant differences between genotypes Bc/- (orientis) and both Bc/bC (‘infumata’) and bC/b- (klugii). Thus, klugii is the larger morph and the hybrid is intermediate. Among Dar es Salaam laboratory-reared males (DB1, Supporting Information, Figs S4B, S5H; Table S5) the heterozygote Bc/bC is significantly larger than its Bc/- (orientis) parent but there is no clear hybrid effect. In wild-caught females from Dar es Salaam (DB2, Supporting Information, Figs S4C, S5I; Table S6), after controlling for seasonal changes in wing length, genotype bC/b- is significantly larger than its putative F₁, Bc/bC. The other putative parental class, Bc/-, is intermediate and cannot be statistically distinguished from other genotypes. However, the size ranking bC/b- (klugii) > Bc/bC (‘infumata’) < Bc/- (orientis) suggests relative unfitness in heterozygous females, which would be in agreement with the Haldane rule. This result is consistent with the breeding data in Supporting Information, Table S9, which show a significant shortage of Bc/bC females in the F₁ in laboratory dihybrid crosses drawn from the same population that was field sampled. The shortfall of Bc/bC females ($\chi^2 = 7.806$, $P = 0.002$) and surplus of Bc/bC males ($\chi^2 = 4.550$, $P = 0.033$) are both significant and suggest heterozygote inviability in females and heterosis in males. Taken together both the wing length and viability data imply that hybrids which are heterozygous at the BC supergene conform to Haldane’s rule.

Among females from wild-collected eggs throughout the hybrid zone (DB3, Supporting Information, Figs S4E, S5K, S, U; Table S7), in the putative cross klugii (bC/b-) × alcippus (bC/bC) the bC/bC (‘transiens’), offspring are significantly different from both parents and intermediate in size. In the putative cross bC/b- (klugii) × Bc/- (orientis) there is a significantly lower wing length in F₁ individuals identified as Bc/bC (‘infumata’) (Fig. 3A; Supporting Information, Figs S4E, S5M; Table S7) compared to both parents. As males (see below) are not similarly affected, this is again consistent with Haldane’s rule. In the putative cross orientis (Bc/-) × alcippus (bC/bC) (Supporting Information, Fig. S5O) the F₁, Bc/bc females are smaller than both parents but significantly so only from the former.

In males (DB3, Fig. 3B; Supporting Information, Fig. S5R, T, V; Table S7) the bC/bc offspring are intermediate between the klugii and alcippus parents, and significantly smaller than the former. In the orientis × klugii cross there are no significant size differences between either parent and their F₁ (Supporting Information, Fig. S5T).
Figure 3. Bateson-Dobzhansky-Muller-like effect at the BC locus (Supporting Information, Table S7) in the presumptive cross *orientis* (*Bc*/*Bc*) × *alcippusichrysippus* (*bc*/*bc*) in wild-collected eggs reared to adult in the laboratory (DB3). In both sexes (A, females; B, males) the F$_1$ heterozygote is smaller than both its homozygous parents and significantly so in all cases except in the comparison female *bc*/*bc* and the F$_1$ *Bc*/*bc*.
the orientis × alcippus cross (Fig. 3B; Supporting Information, Figs S4F, S5P) the Be/bc offspring are very significantly smaller than both putative parents. As both sexes are smaller than their presumptive parents, this is a BDM effect.

As heterozygotes at both B and C loci are only partially identifiable by sight in the field, the statistical conclusions are very conservative. Penetrance estimates obtained from breeding for c in Cc heterozygotes are: in Bc/bc ('transiens'), 0.514 (N = 1063); in Be/bC ('infumata') 0.563 (N = 1209). Penetrance of b in Bb/bc heterozygotes cannot be reliably estimated from sight. As no interactions between A locus effects (Supporting Information, Fig. S5A-F) and BC effects (Supporting Information, Fig. S5G-V) were detected, the two effects are assumed to be additive.

There is ample evidence that the three morphs differ in wing length in the order klugii > orientis > alcippus in both sexes (Table 2). It seems that the BC supergene controls both wing morphology and colour pattern, as is known to be the case for supergenes in Heliconius numata (Cramer), Melinaea (Jones et al., 2013) and Papilio memon L. (Clarke et al., 1968; Clarke & Sheppard, 1971). However, it is not clear from this study to what extent, if any, these differences in wing length might reflect in part either the migratory habit of the butterfly (Smith & Owen, 1997) or parasitism by Spiroplasma (Herren et al., 2007). As both migration and parasitism are well known to affect body size in D. plexippus (Altizer et al., 2000; Altizer & Davis, 2010; Altizer et al., 2011; Dingle, 2014), it is our intention in future studies to investigate the possible influence of both factors in D. chrysippus.

The wild-caught samples from throughout the contact zone (DB4, Supporting Information, Figs S4E-F, S5K-P; Table S8) are controlled for collecting site but not for season. The frequency distributions for forewing length between A locus effects (Supporting Information, Fig. S5G-V) cannot be reliably estimated from sight. As no interactions between BC effects (Supporting Information, Fig. S5A-F) and BC effects (Supporting Information, Fig. S5G-V) were detected, the two effects are assumed to be additive.

DISCUSSION

Here we have used the well described colour pattern loci of the African monarch to test for different hybrid effects both in controlled laboratory crosses and in the presumptive F1 animals captured in contact zones in the field. Strikingly, we find evidence for all three anticipated effects: heterosis (A locus), BDM incompatibilities (BC locus) and sex-specific effects consistent with Haldane’s rule (BC locus) can clearly be seen in different crosses both in the laboratory and more importantly in the field. This leads to several important conclusions. First, by using clearly defined autosomal markers for the separate semi-species we can document hybrid effects in the field that have previously only been seen in the laboratory (Smith, 1980), raising the possibility that similar approaches could be used in other animals and plants. Second, our data suggest that the colour pattern loci affect both colour and body size and that it is these autosomal loci themselves that are largely responsible for the hybrid effects seen.

Whatever the outcome of the ongoing discussion regarding the subspecific divisions of D. chrysippus and their nomenclature (Vane-Wright & John, 2019; Vane-Wright, 2020), the body size differences of hybrid offspring described here, compared to their homozygous parents, are at once atypical for a polymorphism but conform to expectation if disruptive selection is (or has been) underway. Such differences among homozygous parents and their heterozygous progeny are commonplace when hybrids between ‘ecotypes’ that have been selected ecologically in different environments, such as in three-spine sticklebacks, Gasterosteus aculeatus L. (Gow et al., 2007), are adversely selected whenever their parents interbreed. Owen & Chanter (1968) noted that in D. chrysippus in Uganda, Aa hybrids at the A colour gene locus were under-represented in wild populations compared to their presumptive AA and aa parents, again suggesting disruptive selection against heterozygotes.

Table 2. Size comparisons among the semi-species of D. chrysippus, females upper right, males lower left

|                     | bC/b- (klugii) | Be/c (orientis) | bc/bc (alcippus) |
|---------------------|---------------|----------------|-----------------|
| bC/b- (klugii)      | NC            | klugii > orientis*** | klugii > alcippus*** |
| Be/c (orientis)     | NS            | NC             | NS              |
| bc/bc (alcippus)    | klugii > alcippus*** | orientis > alcippus*** | NC              |

***, P < 0.001.
NS, not significant.
NC, no comparison.
These hybrid effect findings have implications for the hypothesis of ongoing speciation among the several forms of *D. chrysippus* in Africa, which occupy substantially different ranges, where each colour form is close to monomorphic. It seems likely that the colour pattern variation in *D. chrysippus* was evolved in allopatry and in response to differing environments (Smith et al., 2016, 2019). As the species is notoriously migratory (Smith & Owen, 1997), especially the males, both sex ratio and morph composition at any single site are in constant flux (Supporting Information, Fig. S2B-E), mostly in response to the north-south oscillations of the ITCZ. Therefore, both polymorphism and sex-ratio variation in a given place are to some extent artefacts created by the high mobility of the species and more rapid dispersal of males. Because all the geographical forms retain the ability to mate and bear fertile offspring in the contact zone, numerous hybrid colour forms occur.

Whereas, on the isolation by distance principle (Wright, 1943), marker colour genes have reached all quarters of the African continent and even far beyond, e.g. Sri Lanka (Vane-Wright, 2020), the part of East Africa we call the contact zone (Smith et al., 2019; Fig. S6) is an area of permanent admixture polymorphism where the frequency of each semi-species varies seasonally (Fig. 2B), in the main caused by migration (Smith & Owen, 1997). This is, emphatically, not to contend that no stragglers will remain outside their usual geographical limits and possibly interbreed with long-term residents within the contact zone. It is within this contact zone that, when hybridizing with neighbouring aliens, sporadically separated semi-species have each left their marks in the form of heterosis, Haldane’s rule and BDM effects. Heterosis occurs in the cross A- (klugii + orientis) × aa (alcippus) (Fig. 1); Haldane effects are associated with the crosses klugii × orientis and orientis × alcippus (Fig. 2); a full BDM effect is found only in the presumptive cross Bc/c (orientis) × bc/Bc (chrysippus + alcippus) (Fig. 3); the latter most, especially, suggests either that incipient speciation is ongoing, or alternatively, that it might have occurred in the recent past and been subsequently interrupted or reversed (Smith, 1980).

An overall interpretation of the various hybrid effects suggests, firstly, that a relatively ancient split occurred between (klugii + orientis) and (alcippus + chrysippus), a divergence which manifests as heterosis and BDM effects in crosses between them. Secondly, a later divergence in East Africa which involved, in particular, *klugii* and *orientis*, is indicated by Haldane effects. Our findings also have implications for understanding the mechanisms underlying Haldane’s rule. There is convincing evidence that Haldane’s rule can usually be explained by the exposure of recessive Z-linked (or X-linked) incompatibilities in the heterogametic sex (Coyne, 2018) — originally coined the ‘large X effect’ (Coyne & Orr, 2004). However, we have here defined ‘hybrids’ purely by being heterozygous at autosomal markers, with no evidence for the population origin of the Z and W chromosomes in the crosses assessed. Moreover, the Z chromosome shows minimal genetic differentiation among semi-species (Martin et al., 2020), implying that it is largely homogenized by gene flow, and is therefore unlikely to harbour BDM incompatibility loci (Bank et al., 2012). Therefore, the reduced hybrid female fitness associated with heterozygosity at the BC supergene could either be explained through an interaction with the W chromosome (which has yet to be sequenced) or must be unrelated to the fact that females are heterogametic. Further work will be needed to resolve this.

Previous studies of all three effects on field populations of animals and plants have relied upon isolating strains from the field and then crossing them in the laboratory. Here we have done the same but have extended our work to test for the actual significance of these interactions in the field itself. Thus, by comparing laboratory crosses of known genotypes with data from presumptive F₁s in the field we have been able to confirm that these effects are still (highly) significant under field conditions.

ACKNOWLEDGEMENTS

Matt McClements (Blink Studios Ltd.) generated the figures. Alven Liao generated the wing images. The following collected field material or assisted with breeding: Matthew Barnes, Lorna Depew, Jeremy Herren, Frank Jiggins and countless undergraduates of the universities of Dar es Salaam (1974–1975), Nairobi (1985–1986) and Rwanda (2017–2019). We are much indebted to Richard Vane-Wright and an anonymous reviewer for their comments on the submitted draft of this paper which have contributed substantially to its improvement.

FUNDING

Funded by a grant BB/H014268/1 to R.ff-C from the Biotechnology and Biological Sciences Research Council or BBSRC

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Wing length xlsx files available on request. Table of all databases used in this study and their associated codes.

Table S2. A, probabilities (p) of difference among wing lengths (mm) of genotypes at the A locus of *D. chrysippus* laboratory-reared on *C. gigantea* at Dar es Salaam (1974-1975). DB2, Supporting Information, Figure 5A-B. B, raw data for Supporting Information, Table S2A.

Table S3. A, probabilities (p) of difference among wing lengths (mm) of genotypes at the A locus of *D. chrysippus* wild caught at Dar es Salaam (1974-1975), DB1, Supporting Information, Figure 5C-D. B, raw data for Supporting Information, Table S3A.

Table S4. A, probabilities (p) of difference among wing lengths (mm) of genotypes at the A locus of *D. chrysippus* wild caught throughout the contact zone, DB4, Supporting Information, Figure 5E-F. B, raw data for Supporting Information, Table S4A.

Table S5. A, probabilities (p) of difference among wing lengths (mm) of genotypes at the BC locus of *D. chrysippus* laboratory-reared on *C. gigantea* at Dar es Salaam (1974-1975), DB2, Supporting Information, Figure 5G-H. B, raw data for Supporting Information, Table S5A.

Table S6. A, probabilities (p) of difference among wing lengths (mm) of genotypes at the BC locus of *D. chrysippus* wild caught at Dar es Salaam (1974-1975), DB1 in part and DB4, Supporting Information, Figure 5I-J. B, raw data for Supporting Information, Table S6A.

Table S7. A, probabilities (p) of difference among wing lengths (mm) of genotypes at the BC locus of *D. chrysippus* wild-collected eggs, laboratory-reared in Nairobi (1985-1986), DB3, Supporting Information, Figure 5K-P. Presumptive crosses are klugii (bC/b-) × alcippus (bc/bc) (SS5-L), klugii × orientis (bC/bc) (SS5-M-N), orientis × alcippus (S5O-P). B, raw data for Supporting Information, Table S7A.

Table S8. A, probabilities (p) of difference among wing lengths (mm) of genotypes at the BC locus of *D. chrysippus* wild caught throughout the contact zone, DB4, Supporting Information, Figures S5Q-V. Presumptive crosses are klugii (bC/b-) × alcippus (bc/bc) (Q-R), klugii × orientis (bC/bc) (SS5-T), orientis × alcippus (S5O-V). B, raw data for Supporting Information, Table S8A.

Table S9. *F₂* offspring (expected numbers in parenthesis) from 13 *Bc/bC × Bc/bC* crosses at Dar es Salaam, Tanzania, 1975. Data from Smith (2014).
Figure S1. Forewing length in *D. chrysippus*. The blue line A-B is the parameter measured. The numbering system for veins and spaces follows Higgins & Riley (1970).

Figure S2. A, seasonal variation in wing lengths of *D. chrysippus* – males (blue) and females (brown). Error bars show two standard errors of the mean. B, seasonal frequencies of A locus genotypes in females. C, seasonal frequencies of A locus genotypes in males. D, seasonal frequencies of BC genotypes in females. E, seasonal frequencies of BC genotypes in males. All the data relate to butterflies wild caught, marked and released on the university campus at Dar es Salaam in 1974–1975. Genotype and phenotype (Table 1) of the butterflies figured are as follows: A, 1.1 ♀; Aa, 2.1 ♀; aa, 3.1. ♂; Bc/bC, 1.6 ♀; Bc/bC, 1.5. ♀; bC/bc, 1.4, ♂; bC/bc, 1.3, ♀; Bc/c, 1.2, ♂; bC/c, 1.1, ♀.

Figure S3. Beanplots showing frequency distributions of forewing lengths (mm) associated with genotypes at the A locus at Dar es Salaam. A, laboratory-reared females (DB2). B, laboratory-reared males (DB2). C, wild-caught females (DB1). D, wild-caught males (DB1). E, wild-caught females, (F) wild-caught males from DB4. C and D controlled for seasonal change, E and F controlled for collecting sites but not for seasonal change.

Figure S4. Beanplots showing frequency distributions of forewing lengths (mm) associated with genotypes at the BC locus. A. Lab-reared females from Dar es Salaam (DB2), controlled for food-plant. B. Lab-reared males from Dar es Salaam (DB2), controlled for food-plant. C. Wild-caught females from Dar es Salaam (DB1). D. Wild-caught males from Dar es Salaam (DB1). E. Wild-caught females from throughout the contact zone (DB4). F. Wild-caught males from throughout the contact zone (DB4). G. Female samples reared from wild-collected eggs (DB3), controlled for food-plant. H. Male samples reared from wild-collected eggs (DB3), controlled for food-plant.

Figure S5. Graphs showing the observed wing lengths of *D. chrysippus* parents and, in laboratory crosses, F₁ hybrids. The sexes are shown separately throughout. In wild populations the F₁ crosses are presumptive rather than actual. Points in blue show, on the left, the mean values of the two parent genotypes and, on the right, the F₁. Mid-parent values are marked as horizontal lines in blue. Statistical significance of size differences: * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. Differences not marked as statistically significant might in some cases be so if sample sizes were larger. A, female and (B) male genotypes, A-, aa (parents) and Aa (F₁) in laboratory-reared crosses at Dar es Salaam, DB2, controlled for food plant. C, female and (D) male genotypes, A-, aa (parental genotypes) and Aa (F₁ genotype) in a wild population at Dar es Salaam, DB1. E, female and (F) male genotypes, A- aa (parental genotypes) and Aa (F₁ genotype) in wild populations through the contact zone, DB3, controlled for collecting site. G, female and (H) male genotypes, Bc/c, bC/bc (parents) and Bc/bC (F₁) in laboratory-reared crosses at Dar es Salaam, DB2, controlled for food plant. I, female and (J) male genotypes, Bc/c, bC/bc (parental genotypes) and Bc/bC (F₁ genotype) in a wild population at Dar es Salaam, DB1. K, female and (L) male genotypes, bC/bc, bC/bc (parental genotypes) and bC/bc (F₁ genotype) in wild populations through the contact zone, DB4. M, female and (N) male genotypes, Bc/c, bC/bc (parental genotypes) and Bc/bC (F₁ genotype) in wild populations through the contact zone, DB3. O, female and (P) male genotypes, Bc/c, bC/bc (parental genotypes) and Bc/bC (F₁ genotype) in wild populations through the contact zone, DB4. Q, female and (R) male genotypes, bC/bc, bC/bc (parental genotypes) and bC/bc (F₁ genotype) in laboratory-reared butterflies reared from wild collected eggs in the contact zone, DB3 controlled for food plant. S, female and (T) male genotypes, Bc/c, bC/bc (parental genotypes) and Bc/bC (F₁ genotype) in laboratory-reared butterflies reared from wild collected eggs in the contact zone, DB3, controlled for food plant. U, female and (V) male genotypes, Bc/c, bC/bc (parental genotypes) and Bc/bC (F₁ genotypes) in laboratory-reared butterflies reared from wild collected eggs in the contact zone, DB3, controlled for food plant.

Figure S6. A, African vegetation at the maximum extent of Pleistocene glaciation, 18 Kya BP. Q, Mount Camaroun; (R) Ethiopian Highlands; (S) Kenya Highlands; (T) Tanzanian Highlands. B, African vegetation from ~15–5 Kya BP in the Holocene, known as the African Humid Period (AHP). A, River Niger; (B) River Benue; (C) River Nile; (D) River Congo; (E) River Zambezi; (F) Araoune Basin; (G) Ténéré Basin; (H) Lake MegaChad; (I) Lake Tana; (J) Sudan Swamp; (K) Lake Turkana; (L) Lake Victoria; (M) Lake Tanganyika; (N) Lake Malawi; (O) Okavango Delta; (P) Lake Zaire. Vegetation maps for former glacial periods would resemble Figure 6A. A vegetation map for the last interglacial (Eemian) Period, 130–115 Kya BP (marine isotope stage 5e), is virtually identical to Figure 6B, as are vegetation reconstructions for many former interglacial and interstadial periods in the Pleistocene.