Polymorphism and evolution in the butterfly *Danaus chrysippus* (L.) (Lepidoptera: Danainae)

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Analysis of the genetic structure of a sample of the polymorphic butterfly *Danaus chrysippus* from Kampala, Uganda shows that the population is undergoing substantial evolutionary change. Comparison with samples from the same area going back to before 1900, indicate that the frequency of form *alcippus* has increased from 16 per cent to 71 per cent (1909–91) while f. *dorippus* has decreased from 14 per cent to 2 per cent, f. *aegyptius* from 66 per cent to 24 per cent and f. *albinus* from 4 per cent to 3 per cent. Genotype frequency differences between the sexes at two of the three loci examined suggest that a balanced polymorphism is maintained by opposing selective forces acting on males and females. Non-gametic (genotypic) disequilibrium between two pairs of unlinked loci indicates that natural selection is involved, again with sex differences. It is suggested that the polymorphism originated after hybridization of allopatric races which evolved during the Pleistocene but are now maintained sympatrically. The selective agents have not been identified but mimetic relationships, both Batesian and Müllerian, are almost certainly involved.

Keywords: allopatric evolution, *D. chrysippus*, mimicry, non-gametic disequilibrium, polymorphism, sympatric evolution.

Introduction

In much of Africa the butterfly *Danaus chrysippus* (L.) (Nymphalidae: Danainae) is polymorphic for colour and pattern. As it is both a Batesian model for several unrelated species, belonging to four different butterfly families (or subfamilies depending on the authority) and also a Müllerian mimic sharing in rings with several other unrelated unpalatable species, it is a remarkable exception to the rule that both Batesian models and Müllerian mimics are monomorphic (Owen, 1971; Smith, 1980; Gordon, 1984). The polymorphism is best developed in East and Central Africa, especially in Uganda; there are also large areas, both within and outside Africa, where the butterfly is monomorphic and this is the general rule, with only local exceptions, throughout its extensive Palearctic, Oriental and Australasian range.

In East Africa there are four main colour forms, still referred to by their traditional Latin names: *aegyptius* (incorrectly called *chrysippus* by many previous investi-

gators in the East African region), *alcippus*, *dorippus* and *albinus* (Fig. 1). Colour plates can be found in Rothschild *et al.* (1975) and Smith (1980).

Many *alcippus* have only a small white patch in the hindwing or simply white scaling lining the veins; these we refer to as weak *alcippus* (also known as *alcippoides*); similarly there are weak *albinus* (also known as *semialbinus*). Many *dorippus* have a variable number of 'aegyptius' subapical spots on the underside of the forewing; these are also called *transiens*. In all four forms the orange ground colour may be completely or partially replaced by brown.

Forms *aegyptius*, *alcippus*, *dorippus* and *albinus* are involved in a Müllerian mimicry complex with *Acraea encedana* Pierre and *A. encedon* (L.) (Acraeinae) and are also models for many presumed Batesian mimics, particularly *Hypolimnys misippus* (L.) (Nymphalidae). Maps showing the distribution of the forms of *D. chrysippus* in Africa are given in Owen & Chanter (1968), Owen (1971), Rothschild *et al.* (1975) and Pierre (1973, 1980). The switch between orange and brown ground colour phenotypes (which have not received Latin names) probably has little impact on

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Fig. 1 Colour forms (f.) of *Danaus chrysippus*. (a) f. *aegyptius*. (b) f. *alcippus*. (c) f. *dorippus*. (d) f. *albinus*. The black and white areas are as shown; the stippled areas are either orange or brown.

generalized mimetic resemblance (Gordon, 1984), especially as many of the mimics are similarly variable; Smith (1980) has suggested that the orange-brown polymorphism relates to climatic adaptation, possibly through thermoregulation, influencing activity levels and, in particular, courtship behaviour.

The genetics of the polymorphism in *D. chrysippus* involves three loci, *A*, *B* and *C* (Smith, 1975a). The *A* locus controls the pattern of the hindwing, the *A* allele giving brown or orange *aegyptius* and *aa* a large white patch (*alcippus*). *Aa* heterozygotes vary between having a reduced white patch, white scaling lining the veins (both scored as weak *alcippus*) or no trace of white (scored as *aegyptius*); hence not all heterozygotes are phenotypically distinguishable from *AA* homozygotes. Estimates for the penetrance of *a* in heterozygotes, based on breeding at Dar es Salaam, Tanzania are 74.4 per cent ($N=47$) (Smith, 1975a) and 65.6 per cent ($N=66$) (Smith, 1980) with an average of 70 per cent. An estimate of 69.7 per cent penetrance for the Kampala sample ($N=211$) suggests good agreement with Dar es Salaam but assumes the alleles are in

Hardy-Weinberg equilibrium, which they are unlikely to be (see below). At Kampala, penetrance of *a* is nearly equal in the two sexes whereas at Dar es Salaam it is 24 per cent higher in males than in females. The *B* locus controls ground colour, *B* giving brown and *bb* orange. Many heterozygotes are detectable but they are variable: *Bb* genotypes always have a brown forewing and a little brown on the costal margin of the hindwing but the hindwing may be entirely brown as in *BB* homozygotes. The *B* locus was first identified by Clarke *et al.* (1973) and confirmed by Smith (1975a). The *C* locus controls the forewing pattern, *C* giving *dorippus* (or *albinus* when combined with a white hindwing), and *cc* giving *aegyptius* (or *alcippus* when combined with a white hindwing). Many *Cc* heterozygotes can be recognized phenotypically by the presence of pale subapical spots on the underside of the forewing (*transiens*). At Dar es Salaam, Tanzania, the expressivity of *c* in *Cc* genotypes is influenced by the *B* locus, 45 per cent ($N=129$) of *bbCc* heterozygotes being *transiens* compared with 74.6 per cent ($N=173$) of *B-Cc* (Smith, 1980).

The *B* and *C* loci are closely linked with a recombination value of 1.9 per cent (3.8 per cent in males only as meiosis is probably achiasmatic in females). The *A* locus assorts independently of *B* and *C* at Dar es Salaam (Smith, 1975a, 1980) and also did so in crosses between *alcippus* (*aa B-*) from Sierra Leone and subspecies *petilia* (*AA bb*) from Australia (Clarke *et al.*, 1973).

Here we report on genotype frequency in a sample of *D. chrysippus* obtained at Kampala, Uganda, in December 1991 and present evidence indicating that powerful natural selection is operating at the present time and that a considerable evolutionary change has occurred in the past 91 years (approximately 1092 breeding generations).

Materials and methods

Source of material

D. chrysippus is an abundant butterfly and large samples are easily obtained by using an ordinary butterfly net. In December 1991 we collected a sample in the Kampala area at sites where previous samples had been obtained by us (1964-66, Owen & Chanter (1968); 1972, A.M.O., unpublished data). We also examined two samples in the Hope Entomological Collections, Oxford, which had been randomly obtained on the instructions of Professor E. B. Poulton. One of these, obtained by C. A. Wiggins in 1909-12, is from the same area as the more recent samples, whereas the other comes from Kome Island (just off

Table 1 Frequency (percentage) of phenotypes and genotypes in a sample of *Danaus chrysippus* collected in the Kampala region, Uganda, in December 1991

Form and colour	Genotype	Male	Female	Population
<i>alcippus</i> , orange	<i>aa bb cc</i>	46.7	24.0	35.5
<i>alcippus</i> , brown	<i>aa B- cc</i>	3.7	7.7	5.7
weak <i>alcippus</i> (= <i>alcippoides</i>), orange	<i>Aa bb cc</i>	26.2	24.0	25.1
weak <i>alcippus</i> (= <i>alcippoides</i>), brown	<i>Aa B- cc</i>	0.9	8.7	4.7
<i>aegyptius</i> †, orange	<i>A- bb cc</i>	15.9	24.0	19.9
<i>aegyptius</i> †, brown	<i>A- B- cc</i>	1.9	5.8	3.8
<i>albinus</i> , orange	<i>aa bb C-</i>	0.9	0.0	0.5
<i>albinus-transiens</i> , orange	<i>aa bb Cc</i>	3.7	0.0	1.9
weak <i>albinus</i> (= <i>semialbinus</i>), orange	<i>Aa bb C-</i>	0.0	1.0	0.5
<i>dorippus</i> , orange	<i>A- bb C-</i>	0.0	1.9	0.9
<i>dorippus-transiens</i> , orange	<i>A- bb Cc</i>	0.0	2.9	1.4
Sample size	<i>A- bb Cc</i>	107	104	211

†Scored as *chrysippus* by some earlier workers, this name should be restricted to the similar but distinct Oriental and Palearctic subspecies.

Entebbe in Lake Victoria), was obtained in 1946, and is included because *D. chrysippus* ranges widely and the colour forms (at a given time) are stabilized over relatively large geographical areas (Owen, 1971). Three earlier large collections, all mainly pre-1900, were examined: two in the Natural History Museum, London and one in the Hope Entomological Collections, Oxford. Although museum samples are notoriously non-random with rare phenotypes generally over-represented, in this case the phenotype frequencies in the three collections are in fact, surprisingly, formally homogeneous and this increases our confidence that they provide a realistic early baseline from which subsequent evolutionary changes are assessed.

Results

Genetic structure of the Kampala population in 1991

Table 1 lists the frequencies of phenotypes and genotypes in the Kampala sample obtained in 1991; Table 2 gives the frequencies of dominant and recessive phenotypes observed at each of the three polymorphic loci: A, B and C. At all three loci only a proportion of heterozygotes is distinguishable from dominant homozygotes and their scores are therefore combined. As the Uganda population of *D. chrysippus* is unlikely to be in Hardy-Weinberg equilibrium, it follows that the estimation of allele frequencies using the observed frequency of recessive phenotypes, which is the only method available to us, must be illegitimate.

There are several factors, known to apply either to the sampled population or to others in East Africa, which are expected to be inimical to the maintenance

Table 2 Frequencies of dominant and recessive phenotypes in a sample of 211 *D. chrysippus* from Kampala, Uganda

Locus	Male		Female		$\chi^2_{(1)}$
	D (%)	R (%)	D (%)	R (%)	
A	44.9	55.1	68.3	31.7	10.820**
B	6.5	93.5	22.1	77.9	9.249**
C	4.7	95.3	5.8	94.2	0.002

D, dominant; R, recessive. ** $0.01 > P > 0.001$ (with Yates' correction).

of a Hardy-Weinberg equilibrium. (i) Selective elimination of *Aa* heterozygotes was observed at Kampala in 1964-66 from a comparison of the proportions of weak *alcippus* among adults reared from wild larvae in the laboratory and in the wild population (Owen & Chanter, 1968). (ii) Sexual selection based on male heterozygote advantage is recorded in Tanzania (Smith, 1975c, 1981). (iii) Assortative mating for both B and C loci is known from Tanzania (Smith, 1984). (iv) Linkage disequilibrium for B and C loci is also present in Tanzania (Smith, 1980). (v) Both primary and secondary sex ratios generally deviate from 1:1 throughout East Africa (Smith, 1975b; I. J. Gordon, unpublished data) and both were low (female biased) in Uganda during 1964-66 (Owen & Chanter, 1968). (vi) Migration has frequently been reported. Thus there are at least six possible factors which either singly or in combination are incompatible with the maintenance of a Hardy-Weinberg equilibrium.

The data (Table 2) show highly significant sex differences for phenotype frequency at the A and B loci: in particular, *aa* (white hindwing) is more frequent in males, and conversely, *A-* (non-white hindwing) in females ($P < 0.01$); at the B locus, *B-* (brown) females and *bb* (orange) males exceed expectation ($P < 0.01$). These effects cannot result from orthodox sex linkage as the A, B and C loci all proved to be autosomal in breeding experiments on populations from Tanzania (Smith, 1975a), Kenya (I. J. Gordon, unpublished data), India (Smith *et al.*, 1988), Sierra Leone and Australia (Clarke *et al.*, 1973). Polymorphisms for sex-autosome translocations, however, cannot be ruled out, at least for the B locus (Smith, 1976b; I. J. Gordon, unpublished data).

In Table 3 we compare the observed A-B and A-C phenotype frequencies using Brandt and Snedecor's formula for Chi-squared. A null hypothesis, that the various combinations are expected to have equal frequencies in each sex, is rejected for both A-B ($P < 0.001$) and A-C ($P < 0.05$) phenotypes. Therefore we conclude that either selective forces causing differential survival or abnormal segregation mechanisms, presumably also maintained by natural selection, are operating to maintain very different phenotype frequencies in the two sexes; moreover, the mechanisms involved affect all three loci investigated, either individually (Table 2) or in combination (Table 3).

The nature of our data precludes further analysis: in particular it is impossible by direct count to quantify

heterozygotes or linkage phases in double heterozygotes, thus ruling out exact tests for linkage disequilibrium. However, chi-squared tests applied to the six data subsets in Table 3 (A-B and A-C for each of males, females and the population), on the null hypothesis that association of genotypes in A-B and A-C combinations is random, are all non-significant. This result suggests there is no linkage disequilibrium between the A and BC chromosomes, either within sexes or in the population as a whole.

On the other hand, some form of disequilibrium is clear from the comparison of phenotype frequency distributions between sexes for both A-B and A-C combinations (Table 3). Among A-B phenotypes, the *AB*, *Ab* and *aB* 'gametes' are all under-represented in males but exceed expectation in females; the opposite applies to the *ab* 'gamete'. The data are not sufficient to determine if the effects at the A and B loci might be additive or interactive but suggest the former. A similar pattern, although involving mainly the A locus, is found in the A-C genotypes; *AC* and *Ac* 'gametes' are more frequent than expected in females whereas *aC* and *ac* are over-represented in males. The A-B pattern suggests some kind of second-order, genotypic (non-gametic or trigenic) disequilibrium within each sex. The sex differences could be caused by abnormal segregation of the type previously recorded at Dar es Salaam (Smith, 1976b) and Nairobi (I. J. Gordon, unpublished data) in which there is some form of association at segregation between autosomes and sex chromosomes, possibly resulting from Y-autosome translocations. As the B and C loci are closely linked (Smith, 1975a), some similarity between the A-B and A-C locus interactions is not unexpected.

Table 3 Observed numbers of A-B and A-C locus phenotypes in each sex of *D. chrysippus* compared with those expected if genotypes are homogeneous between sexes

Genotype	Male N	(%)	Female N	(%)	Totals N
<i>A-B-</i>	3	9.1	15	8.8	18
<i>A-bb</i>	45	51.2	56	49.8	101
<i>aaB-</i>	4	6.1	8	5.9	12
<i>aa bb</i>	55	40.6	25	39.4	80
Totals	107		104		211
$\chi^2_{(3)} = 21.743; P < 0.001$					
<i>A-C-</i>	0	3.0	6	3.1	6
<i>aa C-</i>	5	2.5	0	2.4	5
<i>A-cc</i>	48	57.3	65	55.7	113
<i>aa cc</i>	54	44.1	33	42.9	87
Totals	107		104		211
$\chi^2_{(2)} = 7.678; 0.05 > P > 0.02$					

χ^2 values are calculated using Brandt and Snedecor's formula. *A-C-* and *aa C-* genotypes are amalgamated to avoid low expected values.

Changes in phenotype frequency

Table 4 shows changes in the frequency of *alcippus*, *aegyptius*, *albinus* and *dorippus* in the Kampala area over 91 years, or slightly more, which is approximately 1092 breeding generations, the generation time (egg to egg) of *D. chrysippus* in Uganda being about 1 month. The heterozygote weak *alcippus* (= *alcippoides*) (*Aa cc*) is included under *alcippus* (*aa cc*) and the heterozygote weak *albinus* (= *semialbinus*) (*Aa C-*) is included under *albinus* (*aa C-*); this unfortunate loss of genetic information is unavoidable for reasons given below. Although the three mainly pre-1900 samples are unlikely to be strictly random, and we do not have the exact dates for many of the specimens, they provide a useful baseline against which to measure subsequent changes in phenotype frequency. Moreover, despite the caveats, we are surprised to find that they are formally

Table 4 Comparison of phenotype frequencies of *Danaus chrysippus* around Kampala, Uganda, over 100 years (approximately 1092 generations)

Source	Date	<i>alcippus</i>	<i>aegyptius</i>	<i>albinus</i>	<i>dorippus</i>	N
Natural History Museum (NHM), London	Mainly pre-1900	38.2	27.6	9.2	25.0	76
Rothschild Collection (NHM)	Mainly pre-1900	28.5	40.7	8.7	22.1	172
Hope Department (HD), Oxford	Mainly pre-1900	29.0	34.2	8.5	28.3	272
Wiggins Collection (HD)	1909–12	15.8	66.3	4.0	13.9	101
Kome Island, Lake Victoria (HD)	1946	46.8	38.0	1.3	13.9	79
Owen and Chanter (1968)	1964–66	37.4	49.6	3.6	9.4	530
Owiny	1971–72	56.5	34.8	0.0	8.7	23
Owen, Gordon and Smith	1991	71.1	23.7	2.8	2.4	211

Values are percentages.

homogeneous ($\chi^2_{(6)} = 6.295$; $0.5 > P > 0.3$) and all differ from the more recent ones in similar ways.

As shown in Table 4, the frequency of *dorippus* has declined steadily and consistently over the whole period; *albinus* also declined up to 1946 but has been fairly stable at low frequency since then. The *alcippus* phenotype shows the most dramatic change in frequency. It reached a low point of 16 per cent during 1909–12 and then increased steadily to reach 71 per cent in 1991. The apparent drop in frequency during 1964–66 may not be real as the standard errors for 1946, 1964–66 and 1971–72 overlap.

Figure 2 shows changes in frequency of the *aa* (+ some *Aa*) and *cc* phenotypes for the period 1909–91. We start the graphs in 1900 by averaging the three pre-1900 collections (Table 4). The graph for the *cc* phenotypes (*aegyptius* + *alcippus*) is based on direct observation; the data for the *aa* phenotypes (*alcippus* + *albinus*) are substantially inflated in Fig. 2 (by 30.3 per cent for the 1991 sample) because of the inclusion with the recessive *aa* phenotype of some *Aa* butterflies (weak *alcippus* + weak *albinus*). This error is unavoidable, if the large random sample for 1964–66 and one of the museum samples were included (which is essential), to ensure comparable treatment of all samples: the 1964–66 data (Owen & Chanter, 1968) are for butterflies marked and released and therefore not available for examination. The study was carried out before the genetics of *D. chrysippus* had been fully worked out and all individuals with some white in the hindwing were scored as *alcippus* (or *albinus*).

Regression coefficients are required to quantify with precision the changes of phenotype frequency over the period 1910–91; unfortunately, the small number of data points combined with large variation in sample sizes precludes such treatment. Further analysis has therefore to be limited to comparing phenotype frequencies at the start and end of this period and to examining the regularity of the changes by rank corre-

Fig. 2 Changes in frequency of the *aa* and *cc* phenotypes in *Danaus chrysippus* at Kampala, Uganda, during 1900–91.

lation. For the forewing phenotype, *aegyptius* + *alcippus* (the *cc* phenotype) increased from 81.6 per cent to 94.9 per cent; a *z*-test on these data (arcsine transformed) shows that the change is very significant ($z = 2.697$, $0.01 > P > 0.002$). Moreover, the increase is probably regular (Kendall's $\tau = 1$, $P = 0.017$). Making due allowance for imperfections in the data, the *cc* phenotype increased by an average of 0.16 per cent a year or 0.013 per cent per generation. The white hind-winged phenotype (*alcippus* + *albinus*, including weak (heterozygous) forms) increased even more significantly by 54.1 per cent, from 19.8 per cent in 1910 to 73.9 per cent in 1991 ($z = 7.043$, $P < 0.001$) although in this case the increase cannot be accepted as regular ($\tau = 0.8$, $P = 0.08$). The phenotype increase averages 0.67 per cent a year and 0.056 per cent per generation. If the Hardy–Weinberg equilibrium were assumed (see above), these observations would suggest frequency increases over 81 years of approximately 7 per cent for the *c* allele and, more tentatively, in the order of 40 per cent for the *a* allele.

Sustained changes in allele frequency of the magnitude described could result from either introgression from populations to the west of Uganda, or powerful natural selection, or possibly a combination of both. We are unable to estimate changes at the *B* locus, which was only recognized in 1973 (Clarke *et al.*, 1973) but we expect substantial change to have occurred in view of the interactions between the *A* and *B* loci described above and between the closely linked *B* and *C* loci demonstrated in Tanzania.

Discussion

Substantial negative linkage disequilibrium (repulsion phase chromosomes in excess) between the *B* and *C* loci in *D. chrysippus*, based on breeding experiments, was described at Dar es Salaam (Smith, 1980); this result could not be tested or replicated in our small field sample from Kampala, where the frequency of both dominant alleles (*B* and *C*) is too low. Therefore, although the Dar es Salaam and Kampala populations may yet prove similar in having both linkage and non-gametic disequilibria, on current evidence they differ fundamentally: the former shows strong negative linkage disequilibrium for the linked *B* and *C* loci in both sexes whereas the latter shows some other kind of disequilibrium, which we call genotypic, for both *A* and *B* phenotypes considered alone (Table 2) and for *A-B* and *A-C* combinations of unlinked loci (Table 3). The genotypic disequilibrium takes the form of marked sex differences for genotype frequency. The effect does not result from orthodox sex linkage (Clarke *et al.*, 1973; Smith, 1975a). For all three loci, both genotype frequencies and additive or epistatic interactions with each of the other loci differ significantly between the sexes either at Dar es Salaam, Kampala or both.

As the *a* and *b* alleles are subject to adverse selection in females and positive selection in males, the opposing selection pressures, favouring alternative alleles in the two sexes, must result in balanced polymorphisms at both *A* and *B* loci. The genotypic disequilibria show that the *A*, *B* and *C* locus polymorphisms in *D. chrysippus*, at Kampala as at Dar es Salaam, are interlocked in interactions with each other and with sex, possibly involving multiple feedback effects. Allele frequencies must be maintained by a balance of selective forces, acting on a coadapted gene complex (Lewontin, 1974), which operates differently in the two sexes. We predict that disequilibria of the types described here will in time be established as a general feature of all polymorphic East African populations of *D. chrysippus* but we are aware of the importance of replicating the Kampala and Dar es Salaam results in a large random sample from at least one other area.

The breeding data of Owen & Chanter (1968) at Kampala show some unusual segregation patterns for both *A* and *BC* autosomes and sex chromosomes, in particular the occurrence of all-female broods at high frequency. Similar and more extensive results have been obtained at Dar es Salaam (Smith, 1975a,b, 1976b, 1980) and at Nairobi (I. J. Gordon, unpublished data). Features common to all three areas include distorted sex and phenotype ratios together with interaction between them. The causal genetic mechanisms are still under investigation and remain far from fully understood but we believe that sex-autosome translocations and either nuclear or cytoplasmic lethal genes, at least one of which is male-specific, are involved.

A remarkable feature of our Kampala sample is the symmetry of genotype frequency differences between the sexes, the shortage of particular genotypes in one sex, when tested against the null hypothesis that autosomal gene frequencies are expected to be the same in both sexes, being almost exactly balanced by a surplus in the other (Table 3). We have evidence from Dar es Salaam suggesting that a *Y-BC* autosome translocation may be carried out by some females (the female is heterogametic in *Lepidoptera*), resulting in transmission of the normally autosomal *B* and *C* genes exclusively down the female line in all-female broods (Smith, 1976b). (This explanation was originally rejected in favour of meiotic drive but we now have evidence (unpublished data) to suggest that the all-female broods result from the death of male embryos.) These interpretations are supported by unpublished data from Nairobi (I. J. Gordon) and Mombasa (D. Schneider). Thus we have evidence for the occurrence of all-female broods over a large area in East Africa but from nowhere else.

There is, however, no evidence from Kenya or Tanzania for a sex-autosome translocation involving the *A* chromosome, which could provide an explanation, on similar grounds to that for the *B* locus above, for the considerable sex differences in *A* – and *aa* genotype frequencies at Kampala. Therefore we speculate that a *Y-A* autosome translocation, inherited exclusively by females, mainly involving the *A* allele, could explain the predominance of non-white hindwing in females compared with males at Kampala. It is probable that a combination of *Y*-autosome translocation polymorphisms and sex-specific mortality *in ovo* accounts for at least some of the differing genotype frequencies between sexes that we have described in this paper. Why such sex differences, whatever their origins and mode of inheritance, should have been subject to positive selection over much of East Africa is an open question that might in time be answered in terms of the comparative ecology or behaviour of the

sexes. A clear understanding of the genetic mechanisms involved is obviously essential to further progress and will only be achieved through a breeding programme using Kampala material.

The changes in genotype frequency in *D. chrysippus* at Kampala over the past 90 years (Fig. 2), in particular the increase in frequency of *alcippus*, are similar in magnitude and time-span to the spread of the *carbonaria* allele in the moth *Biston betularia* (L.) (Geometridae) in the U.K. in the nineteenth century (Kettlewell, 1973). The main difference is that the moth has only one generation a year so that the evolutionary change in the butterfly involved approximately 12 times the number of generations, although within a comparable time-scale. The considerable genetic changes described here could result either from immigration and gene flow or entirely from natural selection *in situ*, or from a combination of the two. Our data provide no means of distinguishing all these possibilities, although two of our findings indicate emphatically that natural selection is involved. Firstly, the significant differences in genotype frequency between males and females for two of the three loci investigated indicate that, at individual loci, different genotypes have a selective advantage in the two sexes, a situation that must result in balanced polymorphisms, with equilibria determined by the relative strengths of the opposing selective forces. Secondly, the genotypic disequilibrium within sexes found for two pairs of unlinked autosomal loci constitutes *prima facie* evidence for the operation of natural selection at a higher level than the single gene locus or even the chromosome but rather at the level of the integrated gene complex or the genome (Lewontin, 1974; Gordon, 1987).

Although there is an impressive body of intuitive evidence suggesting that mimicry has been an important element in the evolution of polymorphism in *D. chrysippus* (Trimen, 1887; Poulton, 1912; Owen & Chanter, 1968; Owen, 1971; Gordon & Smith, 1989), it is probable that sexual selection, as found at Dar es Salaam (Smith, 1975b, 1981, 1984), and other factors which we are unable to identify, are also involved, especially in the evolution of sex differences.

There are 157 species of danaine butterflies in the world (Ackery & Vane-Wright, 1984), *D. chrysippus* being the only one with a conspicuous colour and pattern polymorphism covering a substantial geographical area (some other species are polymorphic on a local scale where contact between geographical races creates hybrid zones). The question thus arises as to how the polymorphism originated and how it is maintained in an unpalatable species (Rothschild *et al.*, 1975) that in theory should be monomorphic. Con-

versely, why is *D. chrysippus* monomorphic over the greater part of its very extensive geographical range, for example in West Africa, tropical Asia and Australia?

Form *alcippus* (*aa cc*) is the only form present in tropical Africa west of Cameroun and the simplest hypothesis is that western alleles have been and still are infiltrating central and eastern populations. One of us (D.A.S.S.) has proposed that the present sympatric colour forms are derived from previously allopatric subspecies which were isolated by forest barriers before or during the Pleistocene (Smith, 1980; Smith *et al.*, 1988). *D. chrysippus* is a savanna butterfly and its present wide distribution in Africa must be a relatively recent phenomenon associated with a decrease in the area covered by forest as a result of a drier climate, accelerated by an unprecedented impoverishment of forest vegetation from human activities, especially in the last few hundred years. On this hypothesis there is now hybridization between three until recently allopatric subspecies: *alcippus* (*aa B- cc* and *aa bb cc*) to the west of the formerly vast Chad Basin (Lake Mega-Chad), *dorippus* (*AA bb CC*) in the north-east (Somalia), and *aegyptius* (*AA BB cc*) in the south. The absence of full dominance at all three diallelic loci supports our belief that hybridization is a recent event and unusual segregation for sex and other characters may indicate hybrid dysgenesis.

The hypothesis of an allopatric origin for the East and Central African polymorphism is supported by some persuasive evidence, the most pertinent being the present ranges of *alcippus*, *dorippus* and *aegyptius*, centred respectively in the west, north-east and south of Africa, where the populations remain monomorphic. In the precise form stated by Smith (1980), this hypothesis is undoubtedly a simplification of the true state of affairs. For example, *aegyptius* is by far the most frequent phenotype in Africa north of the Sahara, including the Nile Valley in Egypt and the Canary Islands, whereas *alcippus* is occasionally encountered, usually as weak *alcippus*, in localities as far apart as the Canary Islands (Owen & Wiemers, 1992), Algeria (B. Samraoui, unpublished data) and Egypt (El-Aziz, 1991). The allopatric hypothesis clearly requires refinement as follows. Firstly, as *D. chrysippus* ranges without a break across North Africa and the Middle East to the Indian subcontinent and beyond, the derivation of the North African populations may be from the Asian subspecies *chrysippus*, which differs for several characters from the rather similar form from southern Africa. In recognizing these differences, Talbot (1943) accepted the name *liboria* Hulstaert (1931) for the distinctive race occupying the southern third of the continent. Secondly, the *alcippus* gene

might have infiltrated to North Africa and the Canary Islands from across the Sahara at some time in the recent past when much of the area was dry savanna.

Another of us (D.F.O.) has proposed an hypothesis to account for the maintenance, as distinct from the origin, of the East African polymorphism in terms of Batesian mimicry theory (Owen, 1970). In Batesian mimicry the mimics, unless the models are exceedingly distasteful or toxic (Brower, 1960), must generally be less frequent than their models otherwise predators would learn that butterflies of particular colour patterns are usually palatable and the advantage gained by mimicry would soon be lost. Although Batesian mimicry confers advantages to mimics through deception, it is in general a threat to models as predators must occasionally gain experience on palatable species and thence proceed to attack unpalatable ones (Edmunds, 1974). In East Africa in particular, *D. chrysippus* is a model for a considerable variety of Batesian mimics, several of which (*Hypolimnasia misippus* L., *Pseudacraea poggei* Dewitz, *Mimacraea marshalli* Talbot, *Papilio dardanus* Brown) are themselves polymorphic, with alternative forms mimicking different phenotypes of *D. chrysippus* (and in the latter case, other model species). However, in West Africa there are few mimics; here *D. chrysippus* is monomorphic and the *alcippus* phenotype has only one Batesian mimic, the *alcippoides* phenotype of the female *H. misippus* which is always relatively infrequent (Edmunds, 1969; Smith, 1976a). Thus, on this hypothesis, polymorphism in *D. chrysippus* was envisaged to have arisen sympatrically in areas where the species had become threatened by an overload of mimics; in contrast, where there were few mimics, polymorphism did not evolve.

These two hypotheses can now be dovetailed to provide an explanation both for the origin of the polymorphism and for its persistence over such a large area. Our data indicate a substantial increase in the frequency of *alcippus* in Uganda over the past 100 years, a period during which agricultural activities and forest destruction have provided vastly more suitable habitat for *D. chrysippus*. Indeed, our entire study area would have been under forest only a few hundred years ago. We therefore envisage that *alcippus* might be a relatively recent (post-Pleistocene) invader to Uganda from the west. During this time, say 20,000 years, the *a* allele has reached the east coast of Kenya and Tanzania and spread through the Sudan to the Arabian peninsula and beyond (where its frequency is low), creating a west-east morph-ratio cline. The phenotype recurs, possibly as an independent mutant and sometimes at high frequency, in West Malaysia (Corbet & Pendlebury, 1978) and Sumatra where it forms Müllerian co-

mimicry rings with white hindwinged forms of *Danaus affinis* (Fabricius) and *D. genutia* (Cramer) (Ackery & Vane-Wright, 1984). These authors suggest the possibility that the 'alcippus' pattern is primitive in *D. chrysippus* on the grounds that the *a* allele is both recessive (it is actually only partially so) and is widespread at low frequency in the Oriental Region. Similar white hindwinged forms, however, also occur in races of the closely related *D. gilippus* in the New World and in *D. plexippus* in Hawaii. It follows that recurrent mutation for white hindwing in the genus *Danaus* is an equally feasible explanation.

We envisage that *dorippus* moved into Uganda from its heartland in the north-east at some unspecified but probably recent period and combined with *alcippus* to produce *albinus* (which exists only where *alcippus* and *dorippus* are sympatric) whereas *aegyptius* moved up from the south (and possibly down from the north as well). Similar events probably occurred and are occurring in other parts of the range of *D. chrysippus* where it is now polymorphic. The picture is further complicated by the fact that the species is seasonally migratory, thus promoting gene flow in some parts of its range but apparently not in others.

The polymorphism is probably now maintained sympatrically as suggested by Owen (1970). It is necessary, however, to incorporate the historical hypothesis of allopatric origins with that of sympatric maintenance of the polymorphism to overcome the problem pointed out by Turner (1977) that a novel aposematic mutant in a model species must be at a severe initial disadvantage as predators would fail to recognize it as distasteful. Even a gradual diversification of forms, through racial mingling and hybridization, could threaten the safety of all of them as predators experience increasing difficulty in relating several different images with distastefulness. In particular, there is evidence that the rarer forms of *D. chrysippus* are disproportionately at risk from predation (Smith, 1979). The answer to this paradox may lie in the existence in Uganda of up to four Müllerian co-mimicry rings comprising three sympatric savanna species, *D. chrysippus*, *Acraea encedon* and *A. encedana*, with many of the phenotypes of each species having matching forms in one or both of the others (*A. encedon* also has several non-mimetic forms). In this situation it is possible for a new form, whether mutant or novel immigrant, to join an existing Müllerian mimicry ring and obtain immediate protection. Moreover, if the various matching phenotypes of the three species had evolved by slow convergence in isolated allopatric refuges and all expanded their ranges to overlap and hybridize, more or less in unison and in response to the same habitat changes, this could explain the unique polymorphic Müllerian assemblages

in East Africa. (It is possibly not a coincidence that all three species share a feature, rare in butterflies, unrelated to mimicry and highly suggestive of hybrid dysgenesis, namely female-biased populations with a high frequency of all-female brooding.) Unlike Batesian mimicry, these associations would not be expected to be number-dependent nor would the ranges of the various phenotypes necessarily show a perfect match because all are protected. We are currently investigating the two *Acraea* species to assess the extent of their polymorphic resemblances to different populations of *D. chrysippus*.

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