Evidence From Mitochondrial Dna Supports Earlier Records of African Queen Butterflies (Danaus chrysippus) Migrating In East Africa

EVIDENCE FROM MITOCHONDRIAL DNA SUPPORTS EARLIER RECORDS OF AFRICAN QUEEN BUTTERFLIES (*DANAUS CHRYSIPPUS*) MIGRATING IN EAST AFRICA

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ABSTRACT

Anecdotal evidence has long suggested that the African queen butterfly, *Danaus chrysippus* (L.), is migratory. More recently, records of rapid and seasonal changes in the frequencies of different colour pattern morphs in Tanzania, Ghana, and Kenya have provided further evidence. This paper demonstrates similarly rapid changes in the frequencies of different mitochondrial haplotypes in Nairobi, Kenya over a two-week period. These changes are correlated with changes in the frequencies of homozygous and hybrid colour forms, and are consistent with colour pattern/haplotype associations that have been observed on a continental scale. We conclude that they can only be explained by migration and that mtDNA analysis can be usefully and generally applied in studies of migratory movement.

Old observations and anecdotes accumulated over the past 70 years (Smith & Owen, 1997 and references therein) suggest that some subspecies (figure 1) of the African queen butterfly, *Danaus chrysippus* (L.), may be true migrants, *i.e.* genetically programmed, like the American monarch, *D. plexippus* (Brower, 1977), to undertake long-distance flights that are predictable as to season, route and destination (Williams, 1930). In the case of *D. chrysippus*, the circumstantial evidence is fourfold:

1) in subtropical or warm temperate areas such as southern Europe, the Middle East, Japan, southern Australia, New Zealand and Cape Province, South Africa, the butterfly occurs mainly, or only, in summer;

2) there are many anecdotal records of directional mass movements, some wind assisted, others not (Smith & Owen, 1997);

3) most tropical or subtropical, oceanic islands in the Atlantic, Indian and western Pacific Oceans have been colonized by *D. chrysippus* (Ackery & Vane-Wright, 1984); for example St. Helena in mid-Atlantic has, in addition to *D. chrysippus*, only three species of butterfly *Hypolimnas misippus* (diadem), *Colias croceus* (clouded yellow) and *Lampides boeticus* (long-tailed blue), all ubiquitous migrants (Wollaston, 1879);

4) at Dar es Salaam, Tanzania, and around Nairobi in Kenya, frequencies of the colour forms of *D. chrysippus* (figure 1) change through the year in a predictable, cyclical manner that, at Dar es Salaam, is replicated annually (Smith *et al.*, 1997; tables 1–2).
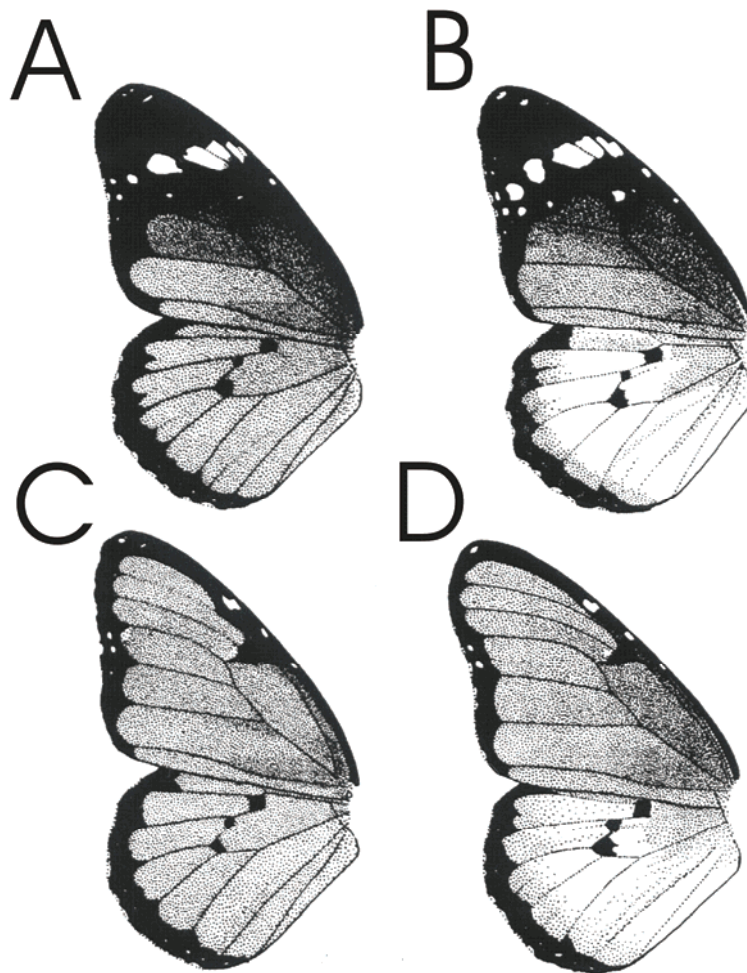

Figure 1. The four principal colour forms of D. chrysippus in Africa: (a) chrysippus, (b) alcippus, (c) dorippus, (d) albinus. The stippled areas are brown or orange; the black and white areas are as shown. Forms chrysippus, alcippus and dorippus are subspecies (figure 2), whereas albinus is a colour form occurring only in the hybrid zone.

D. chrysippus has around seven subspecies arrayed from Africa through Asia to Australia and the West Pacific (Talbot, 1943); however, over a vast area in East and Central Africa the species is polymorphic, *i.e.* several colour forms fly together and interbreed on a regular basis (figure 2). Smith *et al.* (1997, 1998) interpret the extensive areas of polymorphism in Africa as labile contact zones, where the ranges of once vicariant (separated or allopatric) but still inter-fertile subspecies overlap in a seasonally shifting manner. In the past 30 years long-term field investigations of polymorphic *D. chrysippus* populations, by David Smith at Dar es Salaam, Tanzania (1972–75) and by Ian Gordon at Cape Coast, Ghana and at Athi River, near Nairobi, Kenya (1986–95) have revealed sharp seasonal fluctuations of colour form frequencies (Gordon, 1984; Smith *et al.*, 1997). At a single location a colour form may rise to peak frequency in as little as a month or steadily over 3–4 months, followed by a decline. At Dar es Salaam, forms *chrysippus* and *dorippus* have annual cycles of abundance that were recorded continuously over four consecutive years (table 1; Smith *et al.*, 1997). The data from Athi River (table 2) are, in one sense, less convincing as they were collected in six different years (1986–95) and observations were not continuous (see Smith *et al.*, 1997 for details). However, since the butterflies were reared from eggs collected in the field, a possible source of bias was removed compared to samples of adults taken on the wing. According to Cross (2003), each of the three principal colour forms of *D. chrysippus* (figure 1) predominates at a different season in the Wondo Genet Forest Reserve in Ethiopia. In West Africa, *D. chrysippus* form *alcippus* becomes scarce in the coastal belt in the wet season (Ian Gordon & David Smith, unpublished), again suggesting seasonal movement. The question we seek to address is whether these movements in *D. chrysippus* are merely facultative responses to local changes in climate or indicators of cycles that are regularly maintained through varied and irregular seasons.

Table 1. *Seasonal changes in the proportion of* D. chrysippus *that were form* chrysippus *at Dar es Salaam, Tanzania, 1972–75. The remaining butterflies were form* dorippus. *All maxima were in June–July and minima in January–February*

| Year | % frequency form *chrysippus* (N) | | |
	Maxima	Minima	χ^2_1
1972	48.4 (244)	29.6 (54)	5.5‡
1973	36.6 (655)	9.8 (275)	66.8*
1974	31.5 (655)	17.3 (520)	30.0*
1975	35.4 (480)	7.2 (489)	114.3*

N = total number of butterflies scored. *$P < 0.001$, ‡$P < 0.02$.

One explanation for the annual cycles at Dar es Salaam (table 1) is that they reflect alternating migration into and away from the area by the two subspecies *dorippus* and *chrysippus* (figure 1). Similar oscillations in the Nairobi area, involving three subspecies, *dorippus*, *chrysippus* and *alcippus*, probably have the same explanation but the data are less reliable as monthly records are from different years (table 3). However, the amplitude and rapidity of some changes in the frequencies of colour forms at both Dar es Salaam and Athi River were such that Smith *et al.* (1997) considered they must result from population movements rather than cyclical natural selection. That the annual abundance cycles of *chrysippus* and *dorippus* were regularly maintained at Dar es Salaam, despite variation and unpredictability in the alternating wet and dry seasons, supports the migration hypothesis. The Dar es Salaam data (Smith & Owen, 1997), in particular, suggest that dynamic, one-way migration occurs in *D. chrysippus*, unlike the monarch, which exhibits classic two-way

Figure 2. The geographical distribution of the subspecies of D. chrysippus *in the Afrotropical region, showing approximate boundaries of the hybrid zone.*

migration (Brower, 1977; Vane-Wright, 1993). The difference is that *D. chrysippus* would complete the annual 'turn-around trip' over approximately 12 successive, overlapping

Table 2. Seasonal changes in the proportions of the principal colour forms of D. chrysippus *bred from eggs collected at Athi River, near Nairobi, Kenya, 1986–95.*

	Sample sizes (% frequency) of colour forms			
Months	*dorippus*	*chrysippus*	*alcippus/albinus*	*N*
1–2	226 (76.6)*	38 (12.9)	31 (10.5)	295
4–5	220 (75.9)	19 (6.5)	51 (17.6)*	290
7–8	50 (68.5)	11 (15.1)*	12 (16.4)	73
Totals	496	68	94	658

*Months of peak frequency. χ^2_4 = 13.37; *P* < 0.01.

generations, with no reproductive diapause, whereas *D. plexippus* accomplishes the outward journey and first part of the return trip within a single generation. Thus, we envisage that migration by each generation of *D. chrysippus* is unidirectional with different generations taking up successive legs of a bidirectional, or more probably circuitous, annual progression. Many other butterflies fly long distances to places where seasonal, high quality habitat (in terms of e.g. rainfall, day–length or temperature) is predictable; well studied examples are *D. plexippus* (monarch), *Colias croceus*, *Pieris rapae* (small white), *Vanessa atalanta* (red admiral), *Cynthia cardui* (painted lady), *Belenois aurota* (brown-veined white) and *Catopsilia florella* (African migrant) (Williams, 1958; Baker, 1978).

Table 3. DNA sequence variation at diagnostic sites (underlined) in a 347 base pair fragment of the 12S rRNA mitochondrial gene in two samples of D. chrysippus *from Langata, Kenya. Samples 1 and 2 were collected on 23.01.98 and 30.01.98 respectively*

	Diagnostic sites			
	1 1 1 1 1 1 2 2			
12S	3 5 5 6 6 9 0 9	Samples		
Haplotype	3 6 8 1 2 9 0 9	1	2	*N*
ST	– A T T T <u>T</u> A T	5	13	18
GH	– A T T T – – T	0	10	10
NB	<u>T T C A</u> – – – <u>C</u>	10	5	15
Totals		15	28	43

Notation: A = adenine, T = thymine, C = cytosine, – = gap. Haplotypes are ST = standard, GH = Ghana, NB = Nairobi. Minor variant haplotypes at non–diagnostic sites: ST; A at 045 (*N*=1), G at 045 (*N*=1), A at 045 and T at 132 (*N*=1); GH; deletion at 186 (*N*=1), NB, A at 045 (*N*=6), G at 045 (*N*=2), T at 045 (*N*=3), A at 045 and G at 312 (*N*=1), A at 045 and TA at 199–200 (*N*=2), A at 045 and deletion at 186 (*N*=1).

Two samples from a visually polymorphic population of *D. chrysippus* (table 2) at Langata, near Nairobi, were collected with a net in January 1998, seven days apart, in a single 3 ha field of long grass with scattered trees. Both samples were collected in warm, dry conditions in late afternoon. Following DNA amplification with the polymerase chain reaction (see Lushai *et al.*, 2003 for methods), we examined aligned sequences from a 347 base pair (bp) fragment of the 12S rRNA (12S) mitochondrial gene.

We identified three distinct 12S haplotypes based on diagnostic bp differences (table 3), of which two, ST (standard) (*N*=18) and GH (Ghana) (*N*=10), are widespread in Africa and beyond (Lushai *et al.*, 2003). Three ST individuals and one GH were minor variants at non–diagnostic sites (table 3). The third haplotype NB (Nairobi) has so far been recorded only at Langata, where it occurred in 12 butterflies of form *dorippus* and three identifiably hybrid individuals that had one *dorippus* parent (table 3). With six variants at non–diagnostic sites (table 3), NB was more diverse than either ST or GH.

In areas where only one colour form (~subspecies) occurs, ST is the only haplotype found in *chrysippus* from locations as far apart as Malaysia, India, North Oman and Zambia (*N*=30), whereas GH is monomorphic and invariable in *alcippus* from Ghana (*N*=15) (Lushai *et al.* 2003). The ST and GH haplotypes are also significantly associated with their respective colour forms, even in areas such as South Oman, Uganda, Kenya and Tanzania where populations are polymorphic (Lushai *et al.*, 2003). In attempting to demonstrate correlation between haplotype and phenotype, the reader is reminded that the *dorippus* phenotype is variably dominant over both *chrysippus* and *alcippus*; thus, although many

hybrids are identifiable by their intermediate phenotype, *dorippus* individuals with one *chrysippus* or *alcippus* parent may be visually indistinguishable from homozygous *dorippus* (Smith, 1998). However, our data from across the range of *D. chrysippus* show that the 12S haplotype, classified according to the diagnostic sites shown in table 3, is strongly concordant with colour form, ST with *chrysippus*, GH with *alcippus* and NB with *dorippus*, both in monomorphic and polymorphic populations (Lushai *et al.*, 2003, 2004).

There was a significant ($\chi^2_2 = 12.46$; $P < 0.01$) change of haplotype frequency between the two Langata samples, NB predominating in the first and (ST + GH) in the second (table 3). Of the 43 butterflies, 33 were *dorippus* and 10 were intermediate, *i.e.* identifiable hybrid forms, that had one *dorippus* parent, the other being either *chrysippus* or *alcippus* (figure 1) (Smith, 1998). Since the inheritance of haplotype is matrilineal, we estimate that the proportion of butterflies that had *chrysippus* (ST) or *alcippus* (GH) mothers increased from 36.4% in the first sample to 81.8% in the second, and that the change was significant (χ^2_1 (with Yates' Correction) = 4.93; $P < 0.05$). The data in table 3 suggest that the first sample comprised predominantly homozygous *dorippus*, whereas the second was dominated by *dorippus* × *chrysippus* and *dorippus* × *alcippus* hybrids, though many were not phenotypically distinguishable from homozygous *dorippus*. Paradoxically, both *chrysippus* and *alcippus* were missing: reference to the data in table 2 shows that these forms comprised 23.4% of the January–February total for Athi River in 1986–95. As the small Langata (Athi River) collection of 43 butterflies comprised 12 bp variants at 1–2 non-diagnostic sites (table 3), it is clear that haplotype differences between the two samples cannot be ascribed to siblings that had recently eclosed in the area of capture

Whereas mass migrations of the *chrysippus* and *alcippus* forms (figure 1) of *D. chrysippus* have also been observed (Larsen 1978; Samraoui & Benyacoub, 1991), most sightings in East Africa have involved *dorippus*. Notably, four East African records of mass migration (Williams 1930; Smith & Owen, 1997) are all from areas where *D. chrysippus* is polymorphic, and yet not a single *chrysippus* form was seen among thousands of migrating *dorippus*. The significant change in frequency of 12S variants over one week at Langata suggests that *dorippus* butterflies were moving through the region in late January 1998. Our data indicate the potential of mtDNA technology for the study of migration, especially in species such as *D. chrysippus* where, because of hybridisation, matrilineal ancestry may not be identifiable by visible criteria. The hypothesis outlined here could be tested by taking seasonal samples of *D. chrysippus* for mtDNA analysis at different points along the predicted migration routes (Smith *et al.*, 1997; Smith & Owen, 1997), which are predominantly north–south for *dorippus* and *chrysippus* and east–west for *alcippus*.

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