Heteroplasmy of Mitochondrial DNA in the Ophiuroid *Astrobrachion constrictum*

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We demonstrate the presence of mitochondrial heteroplasmy for the cytochrome oxidase I (COI) gene of the brittle star (*Astrobrachion constrictum*). One of the 117 individuals analyzed contained two distinct single-strand conformation polymorphism (SSCP) haplotypes differing by two substitutions; another showed sequence evidence for heteroplasmy. We used polymerase chain reaction (PCR) cloning, SSCP, and sequencing of a 480 bp region of the 5′ end of COI to isolate and characterize these haplotypes. This is the first properly substantiated case of heteroplasmy in an echinoderm species and may have arisen from paternal leakage.

Mitochondrial DNA (mtDNA) has become a powerful tool for assessing relationships among individuals, populations, and species of animals (Avise 1994). As the number of studies using this genome increases, knowledge of the genetics of the genome itself is also increasing. Two of the more surprising discoveries have been the extent of heteroplasmy in animal populations (Lunt et al. 1998) and cases of biparental inheritance of the genome (Gyllensten et al. 1991; Hoeh et al. 1991). Heteroplasmy, the occurrence of more than one type of mtDNA in the same organism, can arise either from mutation of the genome within the individual, heteroplasmy of the original oocyte, or from biparental inheritance. Most published examples of heteroplasmy involve a variation in the number of repeats within the control region of the genome (Lunt et al. 1998). Although the control region is noncoding, it probably contains sequences that initiate replication and transcription (Clayton 1982). In echinoids and vertebrates, the displacement loop (d-loop) structure evidences replication (Matsumoto et al. 1974). The length of repeats found in this region ranges from small microsatellite-like repeats (Wenink et al. 1994) to large repeats of 1100 bp (Wallis 1987). Length heteroplasmy is generally explained by slipped-strand mispairing during replication (Densmore et al. 1985), and high frequencies of length heteroplasmic individuals can occur in some species (Lunt et al. 1998). In a few cases observed heteroplasmy has been attributed to biparental inheritance (Kondo et al. 1990; Magoulas and Zouros 1993). Pa-

Figure 1. **(A)** SSCP gel of COI PCR products from heteroplasmic individual, ER11 (haplotypes A/D), with homoplasmic individuals either side (haplotypes A and D). Other haplotypes found in the Fiordland pop-

70
A GGAAATTGAT TAGTTCCCTT AATGATAGGA GCGCCAGACA TGGCATTCCC TCGAATGAAT AACATGAGAT

Figure 2. COI nucleotide and amino acid sequences for the haplotypes observed in the two heteroplasmic individuals.

replications, or there has been paternal leakage. For the first to occur, heteroplasmy would have to have persisted long enough in the same lineage to evolve two independent mutations.

From studies on *Drosophila* (Solignac et al. 1983) and crickets (Rand and Harrison 1986) it has been suggested that fixation is complete within a few hundred generations. This is a short time for two mutations to have evolved before sorting out into homoplasmic lineages, but without exact knowledge of the sorting out rates and mutation rates within COI of echinoderms we cannot discount this possibility. The second explanation, paternal leakage, seems more plausible. Experiments designed to detect low levels of paternal leakage through repeated backcrossing have shown partial paternal mitochondrial inheritance in *Drosophila* (Kondo et al. 1990) and mice (Gyllensten et al. 1991). These studies suggested that the observed heteroplasmy may be a result of reduced compatibility between egg and sperm due to the use of hybrid strains. However, heteroplasmy attributed to paternal input has been observed in natural

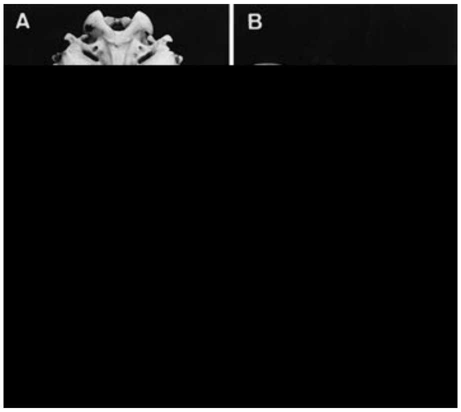

Figure 2. Dental arches of the X trisomy bitch: **(A)** upper dental arch, **(B)** lower dental arch. The missing premolar teeth are indicated by arrows.

curved on both sides instead of being straight. The uterine cavity was filled with a small amount of gray mucus. Ovaries were of normal shape and size.

In the bitch's mouth it was found that some premolar and molar teeth were missing in the upper and lower dental arches three of the eggshell weight estimates. Partial correlation analyses in the two most frequent genotypic classes, *Pst*I(1/1) and *Pst*I(1/2), revealed the presence of a regulatory loop between feed consumption, body weight, egg weight, and the rate of egg laying. Several aspects of this regulatory loop were different between the two genotypic classes. In particular, for the *Pst*I(1/1) genotype, feed consumption was positively associated with egg weight, while there was no significant association for the *Pst*I(1/2) genotype. Further, the degree of association of body weight with egg weight decreased with age in the genotypic class *Pst*I(1/2), while it was constant for the *Pst*I(1/1) genotype. The results indicated that the marker in the IGF-I gene was not only associated with changes in some trait means, but also with changes in the stability of the coordination between feed intake, body weight, and egg production traits.

The components which constitute the growth hormone (GH) axis affect a wide range of biological processes, ranging from growth and differentiation to reproduction (Chase et al. 1998; Feng et al. 1997, 1998; Kocamis et al.1988), immune responsiveness (Aggrey et al.1996; Johnson et al. 1997), and aging (Copras et al.1993; Feng et al. 1997). GH released from the pituitary gland may act directly on target tissues or indirectly by releasing IGF-I from the liver (Isaksson et al.1985). In addition to this major endocrine pathway mediated by the hypothalamus, pituitary gland, and liver, other tissues that produce GH and IGF-I have been identified, indicating that these hormones, together with their receptors and binding proteins, provide a complex regulatory network that coordinates a multitude of traits (Harvey and Hull 1997). Since IGF-I exerts a negative feedback control over GH expression, it is difficult to assign biological effects to either one of the two hormones. Nevertheless, IGF-I is thought to have a direct effect on the interface between nutrient intake and growth (Monaco and Donovan 1997; This-

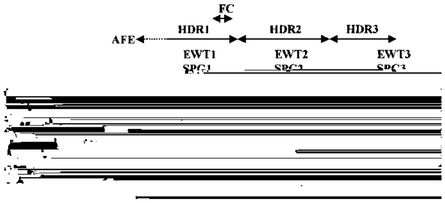

Figure 1. Flow chart of trait measurements. AFE, age at first egg; HDR, rate of egg laying; EWT, egg weight; SPG, specific gravity; HBWT, housing body weight; MBWT, mature body weight; FBWT, final body weight; FC, feed consumption.

Table 1. Association between IGF-I genotypes and traits means

Traits[a]	Significance		IGF-I genotype			Orthogonal contrasts[b] (*P* values)	
	Kruskal–Wallis	ANOVA	*Pst*I (2/2) (*N* = 12–14)[c]	*Pst*I (1/2) (*N* = 83–97)	*Pst*I (1/1) (*N* = 208–248)	Linear (additive)	Quadratic (dominant)
AFE (d)	0.17	0.43	167	167	166	0.808	0.351
HBWT (g)	0.145	0.083	1242	1267	1297	0.160	0.884
MBWT	0.192	0.192	1715	1729	1776	0.320	0.656
FBWT	0.247	0.216	1689	1706	1745	0.327	0.711
HDR1 (%)	0.269	0.140	85.8	85.7	83.7	0.415	0.624
HDR2	0.256	0.365	74.3	70.5	69.2	0.252	0.750
HDR3	0.439	0.576	55.1	56.7	54.3	0.926	0.460
EWT1 (g)	0.052	0.045	50.3[d]	52.8[e]	52.5[e]	0.0[illegible]6	0.023

Table 3. Analysis of variance of factor means[a]

Genotype	N	Factor means[b] F1 (BWT, FC)	F2 (HDR)	F3 (SPG)	F4 (EWT)	F5[c] (HBWT, AFE)
*Pst*I (2/2)	10	20.296	20.113	20.051	20.786[a]	0.357
*Pst*I (1/2)	79	20.138	0.217	0.307[a]	0.102[b]	0.076
*Pst*I (1/1)	201	0.069	20.080	20.118[b]	20.001[b]	20.048
ANOVA (*P* value)		0.222	0.121	0.012*	0.047*	0.557

[a] Multivariate analysis of variance indicated that the differences between genotypes was significant at *P* 5 .0084 (Wilk's lambda, Hotelling–Lawley trace, Pillai's trace) and *P* 5 .006 (Roy's largest root).

[b] Within each column means which differ significantly are marked with different superscripts.

[c] A high score oen5sF6 182c[())-49.89 rai9 Tm(2)Tj/F5 1i5ai9 Tm(2)Tj/F5 1i9,est 24J/F6 11 Tf6.8 0 0 7 26.55 6294rm(2)Ti104t(4J/F64)22(oot).)]TJ/F6 1 Tf4.2 0 0 3.311 Tfl421.05 0 0 3.8 22.55

genotypes. Additional contributions in decreasing order are the factor which represented the rates of egg laying, and the factor which represented body weight plus feed consumption (Table 3).

Partial correlation analyses (i.e., correlation between two traits corrected for the influence of the remaining traits) indicated the presence of a regulatory loop which coordinates body weight, feed consumption, egg weight, and the rate of laying (Figure 3). Thus in the *Pst*I(1/1) genotypic class, heavy chickens tended to lay

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Table 1. Species and DNA sequence used

Species pair	bp	p_b	Number unique	p_w
Genus *Haemonchus*	459	0.152		
H. contortus			37 (50)	0.025
H. placei			31 (40)	0.019
Genus *Teladorsagia*	390	0.134		
T. circumcincta			39 (40)	0.023
T. boreoarcticus			8 (11)	0.005
Genus *Heteror*				

DNA such as this because there are effectively fewer possible character states, so sites saturate quickly (e.g., Brower and DeSalle 1998; Wolfe and Sharp 1993). Unfortunately, how one would accurately correct these tables for multiple hits is not obvious. Any post hoc correction to the tables will need to incorporate a realistic model of the mode of substitution, which in nematode DNA is extremely biased and is not fit by any of the standard models (Blouin et al. 1998).

Further research is needed to determine if selection really acts differently in nematode mtDNA, or if the MK test results are purely an artifact of silent-site saturation. The simplest test would be to find pairs of nematode species that have not diverged as much as these species pairs. Although solved in detail but is regarded as a polyketide synthesis (Cardani et al. 1973). This pathway implies the presence of multifunctional proteins that biosynthesize (part of) the substance through metabolic channeling (Luckner 1990).

After experimental application of *Paederus* hemolymph to human skin, no reactions were observed by Ito (1934) using *P. poweri* and by de Leon (1952) using *P. fuscipes*, two species which were shown by other authors to cause dermatitis (Frank and Kanamitsu 1987). Such a negative result was attributed to immunization of the test person (Théodoridès 1952). Recent chemical analysis of *P. riparius* and *P. fuscipes* (Kellner and Dettner 1995), however, indicate that contradicting evidence using the same species is a real phenomenon due to pederin polymorphism. In both species studied, most of the females accumulate pederin and transfer it into their eggs, whereas some females are obviously unable to biosynthesize the substance and lay eggs without pederin. The former are concisely called (1) females, the latter (2) females. Like the (2) females, larvae and males do not increase their pederin content by themselves but sequester the substance received maternally or consumed if given access to conspecifics.

Polymorphism for a defensive compound is known in great detail from an example in plants: *Trifolium repens* has a cyanogenic and an acyanogenic morph which differ in mollusk acceptability (Dirzo and Harper 1982). Cyanogenesis, the production of HCN, has long been known to be dependent on the presence of cyanogenic glucosides and a specific b-glucosidase (Jones 1972). It is widely accepted that the cyanogenic polymorphism is controlled by alleles of two loci (Hughes 1991): Alleles at locus *Ac* determine the presence or absence of two cyanogenic glucosides, linamarin and lotaustralin, while alleles at locus *Li* regulate the presence or absence of linamarase, a b-glucosidase that hydrolyzes linamarin and lotaustralin. The loci segregate independently according to Mendelian ratios.

After discovering the pederin polymorphism in *Paederus*, Kellner and Dettner (1995) hypothesized that this polymorphism might also be explained by genetic differences. Heterozygous (1) females could then produce homozygous (2) females, which were surmised because some females descended from (1) females had not accumulated pederin when they were analyzed several months after imaginal eclosion. As pederin is present in the hemolymph all the time and not only after liberation by an enzyme after predation as in cyanogenesis, one locus could suffice for the distinction between (1) and (2) females. Analyzing the progeny of known specimens reared in the laboratory, this study aims at finding evidence for or against such a genetic basis of pederin polymorphism.

Materials and Methods

Beetles

Adult rove beetles (*Paederus riparius*) are found in central Europe mainly in spring and autumn (Horion 1965). The beetles reproduce in spring and imagoes of the new generation hibernate (Boháč 1985). Therefore beetles collected in northeastern Bavaria, Germany, from autumn 1992 to spring 1996 were grouped according to their expected season of reproduction, that is, the 1992 autumnal catch was combined with the beetles collected in spring 1993 under the label 1993 and so forth. Nine sites in northeastern Bavaria were visited, some repeatedly, to collect *P. riparius*: two sites in 1993, four in 1994, six in 1995, and two in 1996. The sites lie up to 100 km apart.

In the laboratory the beetles were isolated according to sex and site. Those collected in autumn had to be hibernated artificially by placing them for at least 3 months in a dark climate chamber at 68C. After that period or upon collection in spring, pairs were founded and kept separately as described by Kellner and Dettner (1995) in order to obtain eggs of particular females. The eggs were taken out of the breeding cages three times per week and the larvae reared on moist absorbent paper in 24-cell wells (1.7 cm diameter of the wells). Frozen *Drosophila melanogaster* flies were supplied twice a day. One feeding during each larval stage (first and second stadium) consisted of a piece of either a *Tenebrio molitor* larva or a *Calliphora* pupa, which reduces larval mortality to about 22% (Kellner 1998).

The first-generation laboratory-reared imagoes were kept singly in petri dishes (9 cm diameter) with moist absorbent paper where they were fed with live *D. melanogaster* (strain vg). After artificial hiber-

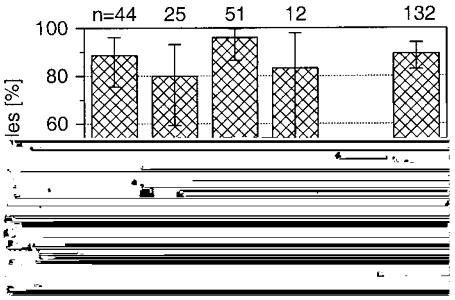

Figure 1. Percentage of (1) females (with 95% confidence interval) in *P. riparius* samples collected from several sites in northeastern Bavaria during four consecutive years. The females were caught during the preceding autumn or in spring of the respective years and laid eggs in the laboratory containing or lacking pederin.

Table 1. (1) and (2) females in the progeny of 17 *P. riparius* (1) females

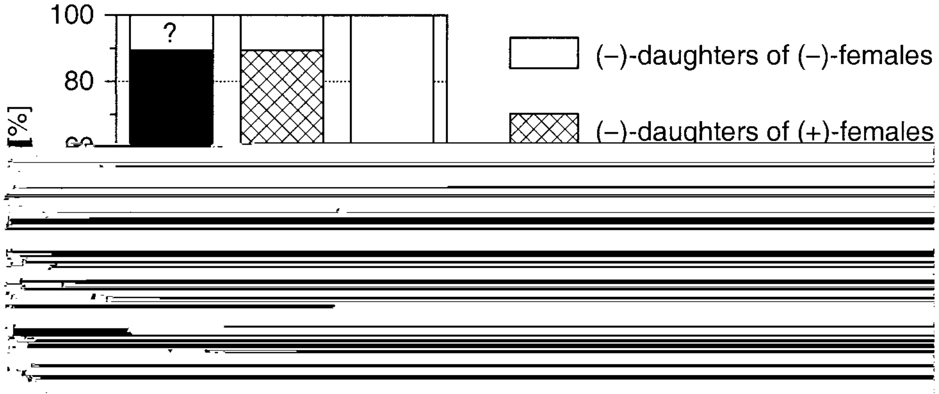

Figure 3. Percentage of (1) and (2) females in successive laboratory generations of *P. riparius*. Collected (2) females (P) are of unknown descent (?).

Table 3. Amount of pederin determined in *P. riparius* (1) females

(1) Females	*n*	Pederin (mg) Mean 6 SEM	 Range
Collected	29	10.8 6 0.62	4.5–16.8
Kept in laboratory	16	12.5 6 1.05	5.3–21.0
Laboratory reared	39	14.7 6 0.92	4.5–24.8

Dead (1

deterred by pederin (Kellner and Dettner 1996) cannot be blamed for that because they reject all progeny of (1) females, that means future (2) females as well. Abiotic factors such as a distinct hibernation rate between (1) and (2) females can be ruled out, as the females collected in autumn and hibernated artificially gave no indication of such a factor's importance.

Regarding the data discussed, it is clear that the initial hypothesis is not supported because the ability to biosynthesize pederin cannot be inherited from the father and furthermore no Mendelian proportions are found in the progeny of (1) mothers. The sudden drop of the percentage of (1) females in F_1

vided support for specific differentiation

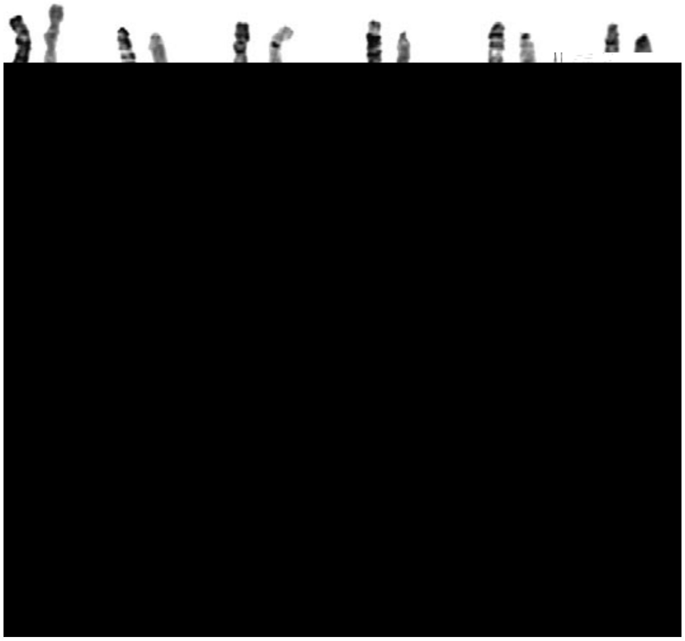

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Figure 1. Composite G-banded (on the left) and C-banded (on the right) karyotypes of a male *P. slevini* (*FN* 5 56). Chromosomes 1, 3, 9, 22, 23, and the sex pair are submetacentric or metacentric; the remaining chromosomes are acrocentric. Heterochromatin is restricted to the centromeric region and the sex pair.

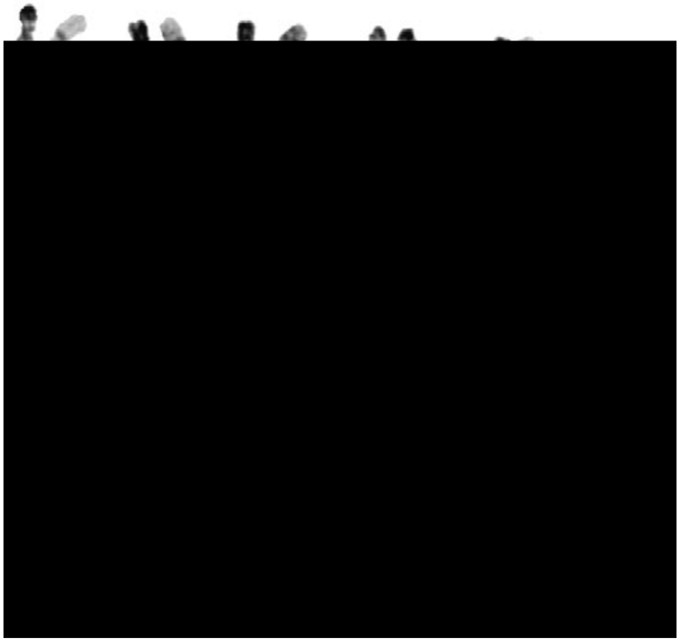

Figure 2. Composite G-banded (on the left) and C-banded (on the right) karyotypes of a male *P. sejugis* (*FN* 5 76). With the exception of chromosome 13, all individuals had indistinguishable karyotypes. Karyotypes from specimens collected on Isla San Diego (the pair on the left) exhibited an alternate form of the submetacentric chromosome 13, designated 13B9. Karyotypes from individuals from Isla Santa Cruz (the pair on the right) have a heterochromatic addition to that chromosome, 13B9 1. For all individuals, noncentromeric heterochromatin is present on chromosomes 11, 18, and 21.

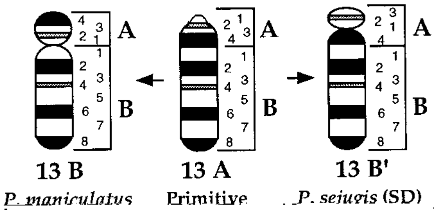

Figure 3. Ideogram of various chromosome 13 conditions within the genus *Peromyscus*. The primitive chromosome 13 is the acrocentric condition. The derived biarmed chromosome 13 in *P. maniculatus* (13B) comes from a pericentric inversion between bands A4 and B1. A pericentric inversion between bands A3 and A4 results in the alternative biarmed condition (13B9) found in karyotypes of *P. sejugis* from Isla San Diego (SD). The presence of heterochromatin on the euchromatic short arm of the latter chromosome forms the 13B9 1 condition indicative of karyotypes of *P. sejugis* from Isla Santa Cruz (not shown).

ated with the *P. maniculatus* species group. Excluding *P. slevini,* the taxa in the *P. maniculatus* species group share indistinguishable biarmed inverted and derived conditions of chromosomes 2, 3, 9, and 20, with the latter character state occurring only in this group and convergently in one cytotype of *P. leucopus* (Stangl 1986). The karyotype of *P. slevini* exhibits acrocentric conditions of both chromosomes 2 and 20. Further, the composite array of chromosomal conformations in the karyotype of *P. slevini* is unique among all reported G-banded karyotypes of deer mice; no other species of *Peromyscus* is known to exhibit the combination of an acrocentric chromosome 2 and biarmed chromosomes 3 and 9.

Although comparisons of the G-banded karyotypes among species of *Peromyscus* do not yield an unambiguous species-group association of *P. slevini,* these data do provide initial hypotheses for studies designed to resolve the phylogenetic position of this species. The karyotype of *P. slevini* is most similar to those that generally characterize taxa in the *P. boylii* and *P. mexicanus* species groups. From the *FN* 5 52 karyotype (biarmed chromosomes 1, 22, and 23) typical of *P. boylii, P. banderanus,* and *P. crinitus,* the karyotype of *P. slevini* differs by having biarmed chromosomes 3 and 9. Compared to the *FN* 5 58 karyotype (biarmed chromosomes 1, 2, 3, 9, 22, and 23) of *P. mexicanus*-group species, the karyotype of *P. slevini* differs by the acrocentric condition of chromosome 2. From the cladistic-based assumption (Rogers et al. 1984; Smith 1990; Stangl and Baker 1984) that the acrocentric condition of chromosome 2 is plesiomorphic for *Peromyscus* and predates the inversions which result in the biarmed conditions of chromosomes 3 and 9, an equal number of inversion events would be needed to explain the differences between the karyotype of *P. slevini* and those of the *P. boylii* and *P. mexicanus* groups, respectively. Cranial similarities of the supraorbital shelf (Carleton 1989), however, support the phylogenetic association of *P. slevini* and the *P. mexicanus*-group as the more likely hypothesis.

The karyotypes of *P. sejugis* from both islands exhibit *FN* 5 76 but are distinguished by the presence of distal heterochromatin on the short arm of chromo-

Isla San Diego provide a character state which would establish *P. sejugis* as a phylogenetic species (see Nixon and Wheeler 1990 and references therein) and support the morphologically based specific distinction of this taxon relative to *P. maniculatus* (Burt 1932). Based on the apparent alternate fixation for 13B9 and 13B91 conditions, a similar argument could be made for a phylogenetic species-based distinction of the two island populations of *P. sejugis*. However, considering the lack of morphologic (Burt 1932), allozymic (Avise et al. 1979) and molecular (Hogan et al. 1997) divergence between these populations we see little value in recommending revision of their current taxonomy.

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The Rift Valley Complex as a Barrier to Gene Flow for *Anopheles gambiae* in Kenya: The mtDNA Perspective

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Descriptions of *A. gambiae* population structure based on microsatellite loci and

Table 1. Polymorphism at the 599 bp segment of the mitochondrial *ND5* gene of *A. gambiae* populations

	Asembo		Jego	
Collection year	1987	1994	1987	1996
N (haplotypes)				
Haplotype diNof				

Table 2. Differentiation between samples from different localities and time points

	Unweighted F_{ST}	Weighted F_{ST}
Asembo 87 vs. Jego 87	-0.041^{NS}	-0.001^{NS}
Asembo 94 vs. Jego 96	0.123***	0.176***
Asembo vs. Jego (pooled)	0.089***	0.142***
Asembo 87 vs. Asemo 94	0.026^{NS}	0.018^{NS}
Jego 87 vs. Jego 96	-0.016^{NS}	0.005^{NS}

*** $P < .001$.

^{NS} $P > .05$.

Table 3. "Lack-of-fit test" with the mutation-drift model using comparison of haplotype F_{ST} and individual F_{ST} (calculated with weights according to sample sizes)

Populations (sample sizes)	Haploytpe F_{ST} (P estimated by permutation test)	Bootstrapped[b] individual F_{ST} (95% CI)
Asembo 94 vs. Jego 96 (25/8)	0.061 ($P > .13$)	0.171*** (0.072–0.285)
Asembo vs. Jego pooled (32/12)	0.058 ($P > .10$)	0.138*** (0.070–0.209)
Senegal vs. western Kenya[a] (17/16)	0.046 ($P < .029$)	0.073*** (0.031–0.134)

*** $P < .001$.

[a] Data from Besansky et al. (1997).

[b] Sample sizes were the same as used for the haplotype F_{ST} calculation (shown in first column).

tween localities in the pooled (over time) data. This level of differentiation and derived estimates of gene flow measured by mtDNA, which were adjusted to the difference in N_e between markers, closely agreed with those measured by microsatellites (Lehmann et al. 1998, 1999). The temporal variation between samples taken 7 (Jego) and 9 (Asembo) years apart from the same localities was minimal (Table 2), suggesting that the difference between the original (Besansky et al. 1997) and the present studies were not due to temporal changes in allele frequencies, nor that the 2 years separating the samples from Asembo (1994) and Jego (1996) contributed much to the differentiation between localities. It can be concluded therefore that the discrepancy between the results based on microsatellites and the original mtDNA study was merely a small sample size effect.

The lack of unique alleles in eastern populations and higher F_{ST} than R_{ST} values measured at nine microsatellite loci suggested that pure drift was the main process generating differentiation between these populations (Lehmann et al. 1999). To distinguish between pure drift and mutation-drift using mtDNA data, a test was developed based on the fact that pure drift affects haplotype frequencies but does not systematically affect the number of pairwise substitutions between haplotypes. Accordingly, if two populations became isolated from each other a few generations ago, and one population has experienced a bottleneck and lost several alleles as part of the rapid change in allele frequencies, then allele frequencies will differ markedly between these populations, but the average mutational distance between two different alleles is expected to be the same, regardless of whether they were taken both from a single population or each from a different population. Independent mutations, in addition to drift, must occur in each population to increase the expected mutational distance between two different alleles, each sampled from one population. Therefore we calculated F_{ST} on haplotypes instead of individuals (haplotype F_{ST}), which estimates the between-population variation in the number of substitutions (i.e., mutations) per haplotype disregarding the haplotype frequency. If differentiation was generated by pure drift, then the haplotype F_{ST} is expected to be zero. Permutation and bootstrapping tests were used to determine the significance of the results and to evaluate whether an insignificant haplotype F_{ST} reflects low statistical power due to smaller sample size.

The haplotype F_{ST} was calculated between Asembo 1994 and Jego 1996 samples, and in the pooled (over time) samples (Table 3). Haplotype F_{ST} values were approximately one-third of the corresponding individual F_{ST} values and were not significant ($P > 0.09$, permutation test), suggesting a lack of fit with the mutation-drift model. To verify that the lack of significance was not a result of weak statistical power due to smaller sample size, we calculated the 95% confidence interval (CI) of individual F_{ST} by bootstrapping over individuals from each population while using the same sample sizes as used for the haplotype F_{ST} calculation. The bootstrapped F_{ST} values were nearly identical to the original individual F_{ST} values (Table 2) and they were significantly higher than zero. Moreover, their lower 95% confidence limits were higher than the corresponding haplotype F_{ST} (Table 3), indicating that the lack of significance of the haplotype F_{ST} values was not due to reduced sample sizes.

In contrast to Kenyan populations across the Rift Valley, higher R_{ST} than F_{ST} values were measured between western Kenya and Senegal (6000 km apart, both west of the Rift Valley barrier) and unique alleles were observed in each population (Lehmann et al. 1996b), suggesting that differentiation between these populations was generated by the mutation-drift model. To test this interpretation, we analyzed the mtDNA data of these populations (from Besansky et al. 1997). Haplotype F_{ST} for this comparison was significantly larger than zero, and it was not significantly different from the individual F_{ST} based on

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Ovis evolved from a common evolutionary pathway. All arose from ancestral stock that shared a $2n = 60$ diploid chromosome number and a karyotype with 29 acrocentric chromosomes, a large acrocentric X, and a very small acrocentric or metacentric Y chromosome. The primitive-type karyotype is still maintained universally in *Capra* and has been maintained during the evolution of the wild and domestic goat and the ibex and markhor. The largest goat acrocentric autosome was involved in the first centric fusion or Robertsonian translocation and became biarm 1q in *Hemitragus, Ammotragus, Pseudois,* and *Ovis* lineage. The pathway for a common 1q arm was shared only in *Ammotragus* and *Ovis*. The fact that both genera share acrocentrics 1q and 3q in the evolution of the first biarmed chromosome suggests they share a common ancestor that arose after a split from goat stock. The *Ammotragus* and *Ovis* karyotype with a $2n = 58$ is shared by the Barbary sheep (*Ammotragus lervia*) and in what is considered the more primitive extant species of wild sheep, the urial (*Ovis vignei*). Based on chromosome fusions involving acrocentric autosome 1 in the *Capra* karyotype, *Hemitragus* and *Pseudois* would have split off from ancestral *Capra* stock separately from *Ammotragus* and *Ovis*.

It is not known which centric fusion occurred first in *Pseudois*. In all likelihood it may have been the 27p/1q translocation, since the 1q was the first centric fusion involved in the $2n = 58$ karyotypes of *Ammotragus* and *Ovis*. The karyotype of the Greater form, Sebei blue sheep ($2n = 56$), has 27p/1q and 29p/2q fusions. Assuming that karyotype evolution of *Pseudois* is toward the reduction of diploid number, then the 14p/5q fusion is the more recent translocation, although it is arranged first in the karyotype of Figure 2 because of its relative size being the largest of the biarmed chromosomes.

Chromosome evolution in Caprini may have set the stage for genetic isolation, which eventually led to speciation. Considering the potential chromosomal segregational problems during meiosis in F_1 hybrids with partial homology of biarmed chromosomes, fertility would be reduced if not totally impaired. F_1 hybrids resulting from a blue sheep ram and domestic goat ewe have been reported at the Henry Dorley Zoo, Omaha, Nebraska, although they were born dead (Bunch et al. 1978). No successful hybridization has been reported between blue sheep and true sheep.

Despite the divergent chromosome evolution in *Hemitragus*, *Ammotragus*, *Pseudois,* and *Ovis,* homologous G-banding patterns in all taxa examined by us and others indicate a conservatism in linear

Table 4. Segregation for seed coat texture in different populations of the cross involving IT93K-693-2 and IAR-1696

	No. of plants with			
Population	Smooth seeds	Rough seeds	x^2	Probability
IT93K-693-2	—	24	—	—
IAR 1696	—	17	—	—
F_1	26	—	—	—
F_1 3 IT93K-693-2	9	7	0.25 (1:1)	.5–.7
F_1 3 IAR 1696	35	32	0.13 (1:1)	.7–.8
F_2	46	37	0.02 (9:7)	.8–.9

the plants of both parents had rough seed coats, but the F_1 plants had smooth seed coats and brown color, indicating independent gene action for seed coat texture and complete dominance for the brown color (Figure 1). The backcross F_1 population involving IT87D-941-1 segregated into 26 smooth-seeded and 23 rough-seeded plants, and the backcross F_1 population involving Kanannado segregated into 21 smooth-seeded and 24 rough-seeded plants, both fitting closely to a 1:1 ratio. The F_2 population segregated into 138 smooth-seeded and 126 rough-seeded plants, showing close fit to a 9:7 ratio. These data indicate that rough seed coat is controlled by two independent recessive gene pairs, and the recessive gene pair for rough coat in IT89KD-941-1 is different from the gene in Kanannado.

Cross 3: Rough 3 Rough

This cross involved a brown-rough-seeded variety, IT93K-693-2, and a white-rough-

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Figure 2. Seed coat texture and color of **(A)** IT93K-693-2 (brown-rough), **(B)** IAR 1696 (white-rough with black hilum), and **(C)** their F_1 hybrid (black-smooth).

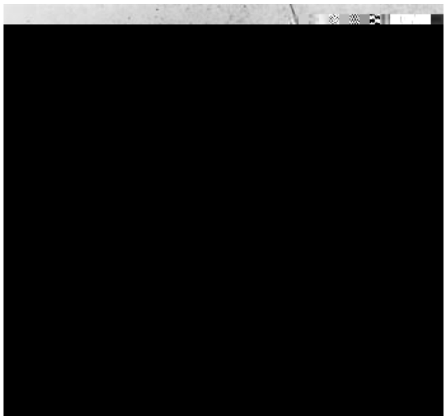

Figure 1. Canide A1AT phenotypes demonstrated by isoelectric focusing and immunoblotting. The bands are the stained immunoprecipitates of alpha 1 antitrypsin. Anode (1) is at the top. The types are 1:M, 2:MS, 3:M, 4:MS, 5:M, 6:S, 7:M, 8:M, 9:S, 1O:S. Samples in the following lanes are from the various canides: 1–3: gray wolf, 4–6: domestic dog; 7 and 8: Mexican wolf; 9: coyote; 10: red wolf.

and Pi^{S} for the common and the slower electrophoretic type, respectively. The phenotypes (band patterns) are called PiM, PiS, and PiMS.

Canine A1AT was isolated as described

Table 1. A1AT (Pi) phenotypes

Group	*n*	M	MS	S
Domestic dog	71	40 (56%)	22 (31%)	9 (13%)
Gray wolf	29	11 (38%)	17 (59%)	1 (3%)
Mexican wolf	20	16 (80%)	4 (20%)	
Red wolf	27			27 (100%)
Coyote	24			24 (100%)
Wolf-dog cross	9	4 (44%)	5 (56%)	

Table 2. Concentrations of A1AT (in mg/ml) 6 standard deviation

Group	Male	Female	Combined
Domestic dog	2.19 6 0.38	2.65 6 0.42	2.42 6 0.41
Gray wolf	2.14 6 0.18	2.34 6 0.35	2.26 6 0.30
Wolf-dog cross	2.28 6 0.21	2.45 6 0.34	2.37 6 0.32
Mexican wolf	2.70 6 0.15	3.06 6 0.11	2.80 6 0.22
Red wolf	2.70 6 0.40	3.04 6 0.32	2.88 6 0.42
Coyote	2.66 6 0.40	2.88 6 0.38	2.72 6 0.43

strated in domestic dogs is also present in gray wolves and that allele frequencies are statistically similar. Mexican wolves are also polymorphic, although the significantly lower Pi^S frequency suggests that they represent a separate population. Red wolves and coyotes are monomorphic for Pi^S. It is as yet undecided if this is due to extensive hybridization or results from common ancestry. Comparison of A1AT concentrations demonstrates the similarity of all animals tested. In addition, the quantitative dimorphism of males and females, known to be present in domestic dogs, was also found in all canids presently tested.

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