related to extant *Pongo*. Meanwhile in Africa there remained a morphotype similar to *Dryopithecus* but with a primitive zygomatic (like *Pan*), which could have given rise to the clade of extant African apes and humans, initiating the development of klinorhynchy. But in the upper Miocene of Africa there exists an enormous fossil lacuna, which effectively renders the origin of the African ape/human clade matter of ongoing uncertainty. □

Received 14 April; accepted 15 July 1993.

1. Begun, D. *Science* **257,** 1929–1933 (1992).
2. Dean, D. & Delson, E. *Nature* **359,** 676–677 (1992).
3. Andrews, P. *Nature* **360,** 641–646 (1992).
4. Lartet, E. *CR Acad. Sci. P.* **43,** 219–223 (1856).
5. Napier, J. R. & Davis, P. R. in *Fossil Mammals of Africa* (British Museum Natural History) **16,** 1–69 (1959).
6. Saban, R. *Mem. Mus. Nat. d'Hist. Nat.* **29,** 1–378 (1965).
7. Pilbeam, D., Rose, M. D., Barry, J. & Shah, S. M. I. *Nature* **348,** 237–239 (1990).
8. Simons, E. L. & Pilbean, D. R. *Folia Primatol.* **3,** 81–152 (1965).
9. Aiello, L. & Dean, C. *An introduction to Human Evolutionary Anatomy* 1–596 (Academic, London, 1990).
10. Schwart, J. H. *J. hum. Evol.* **19,** 591–605 (1990).
11. Andrews, P. & Cronin, J. E. *Nature* **308,** 541–546 (1982).
12. Bonis, L., Bouvrain, G., Geraads, D. & Koufos, G. *Nature* **345,** 712–714 (1990).
13. Groves, C. P. & Patterson, J. D. *J. hum. Evol.* **20,** 167–183 (1991).

ACKNOWLEDGEMENTS. We thank P. Andrews, L. Martin, R. D. Martin, J. Morales and M. Pickford for their comments. We are grateful to the Diputació de Barcelona, DGICYT (PB90-0575) and the Generalitat de Catalunya for their financial support.

A role for central vasopressin in pair bonding in monogamous prairie voles

James T. Winslow*, Nick Hastings†, C. Sue Carter†, Carroll R. Harbaugh* & Thomas R. Insel*‡

* Laboratory of Neurophysiology, National Institute of Mental Health, PO Box 608, Poolesville, Maryland 20837, USA
† Department of Zoology, University of Maryland, College Park, Maryland 20742, USA
‡ To whom correspondence should be addressed

MONOGAMOUS social organization is characterized by selective affiliation with a partner, high levels of paternal behaviour and, in many species, intense aggression towards strangers for defence of territory, nest and mate[1,2]. Although much has been written about the evolutionary causes of monogamy, little is known about the proximate mechanisms for pair bonding in monogamous mammals[2,3]. The prairie vole, *Microtus ochrogaster*, is a monogamous, biparental rodent which exhibits long-term pair bonds characterized by selective affiliation (partner preference) and aggression[4,5]. Here we describe the rapid development of both selective aggression and partner preferences following mating in the male of this species. We hypothesized that either arginine-vasopressin (AVP) or oxytocin (OT), two nine-amino-acid neuropeptides with diverse forebrain projections, could mediate the development of selective aggression and affiliation. This hypothesis was based on the following observations: (1) monogamous and polygamous voles differ specifically in the distribution of forebrain AVP and OT receptors[6,7]; (2) AVP innervation in the prairie vole brain is sexually dimorphic and important for paternal behaviour[8]; (3) central AVP pathways have been previously implicated in territorial displays and social memory[9,10]; and (4) central OT pathways have been previously implicated in affiliative behaviours[11]. We now demonstrate that central AVP is both necessary and sufficient for selective aggression and partner preference formation, two critical features of pair bonding in the monogamous prairie vole.

To characterize the development of selective aggression in prairie voles, sexually naive adult males were tested in a resident–intruder paradigm similar to that used with mice[12]. Naive males, that is, males without female exposure, explored a novel male intruder but showed little attack behaviour. Within 24 h of mating, males showed a qualitative change in their behaviour towards an intruder, with vigorous attacks and threats and decreased defensive behaviour (Fig. 1). This increase in aggression seemed to be sustained and selective. Either with or without further exposure to a female, males continued to show attack

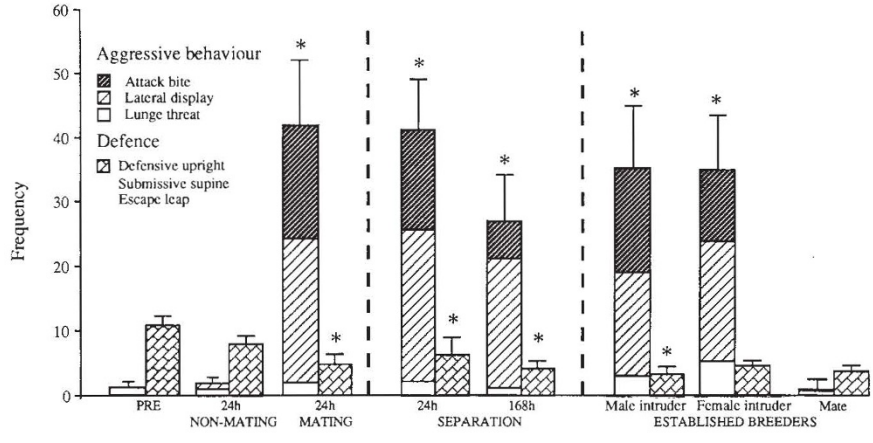

FIG. 1 Mean (±s.e.m.) frequency of aggressive and defensive behaviour in 6-min resident–intruder tests. Before exposure to a female (Pre), sexually naive prairie vole males ($n=17$) exhibit minimal aggressive and moderate defensive behaviour towards a novel male intruder. Tested 24 h later, a subset of these same males ($n=8$) show a similar response to an intruder if housed in the interim with a female without mating (Non-mating). Another subset ($n=9$) that mated with females (Mating) exhibit a significant increase in aggression ($F(6,48)=7.44$, $P<0.05$) when tested after 24 h with the female (Dunnett's t-test, $t_D=3.97$, $P<0.05$) or after subsequent separation from their mates for 24 h ($t_D=4.10$, $P<0.05$) or 168 h ($t_D=2.61$, $P<0.05$). These same males respond to an intruder with comparable levels of aggression after being reunited with their mates for at least 1 week (Established breeder) ($t_D=3.28$, $P<0.05$). Aggression seems to be selective in that it is directed at both males and novel females, but rarely at the mate. A concurrent decrease in defensive behaviour is observed post-mating ($F(6,48)=3.89$, $P<0.05$). An asterisk signifies difference ($P<0.05$) from baseline frequency (Pre) in composite score of aggressive or defensive behaviour by within-subjects *post-hoc* Dunnett's t-test.

METHODS. For aggression testing, socially reared males (60–75 days old) were isolated for at least 3 days to avoid potential effects of social status in communal cages. For the resident–intruder model, an intruder male was placed in the home cage of the experimental animal for 6 min. Each male was tested at baseline and then tested with a different intruder for subsequent tests. For groups with female exposure, the female was removed before adding the intruder male. Behaviour was recorded on videotape and scored using a computer-assisted data acquisition system by a rater blind to group assignment. Behaviour was operationally defined as previously described[22] with reported means indicating frequency over the 6-min test.

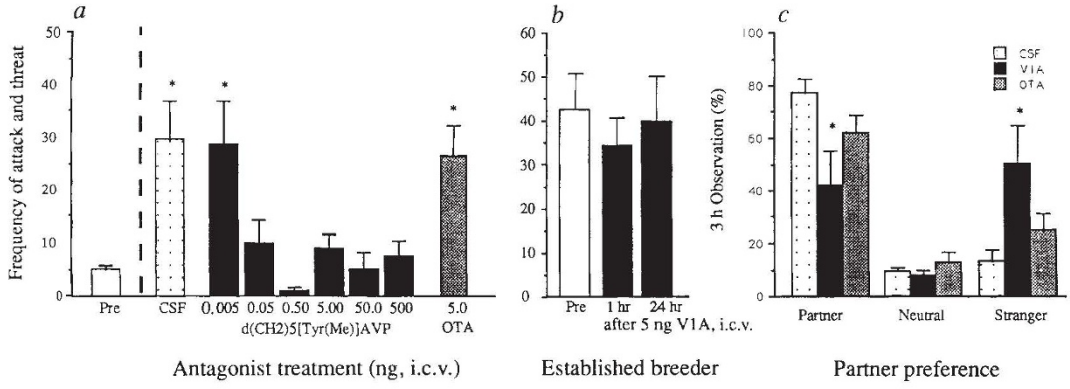

FIG. 2 A V_{1a} antagonist given i.c.v. before mating blocked the induction of aggression in the male prairie vole. *a*, Before female exposure, males showed low levels of aggression (attacks and threats) in the resident–intruder paradigm as described for Fig. 1. Aggression increased after 24 h with a sexually receptive female if the male received i.c.v. injection of CSF, the OT antagonist OTA, or the lowest dose (5 pg) of V_{1a} antagonist d$(CH_2)_5$[Tyr(Me)]AVP ($F(7,54)=4.68$, $P=0.0004$; CSF: Newman-Keuls *post-hoc* comparison, $q_r=8.97$, $P<0.05$; 5 pg OTA: $q_r=8.25$, $P<0.05$; 5 pg V_{1a}: $q_r=8.33$, $P<0.05$). Males receiving all other doses of antagonist failed to increase aggressive behaviour above baseline frequency. Antagonist injection did not alter the number of males mounting females in the first 6 h (χ^2(d.f.$=7$)$=2.186$, $P=0.945$; range$=62.5$–83.3%) or frequency of mounts ($F(3,19)=0.82$, $P=0.50$). *b*, When given to breeder males with established aggression at baseline, d$(CH_2)_5$[Tyr(Me)]AVP (5.0 ng, i.c.v.) had no effect on the number of attacks or threats ($F(2,20)=0.227$, $P=0.80$). *c*, After 24 h of mating, a partner preference was observed in males injected with either CSF ($t=6.71$, $P=0.0005$) or OTA ($t=3.061$, $P=0.03$), but not in d$(CH_2)_5$[Tyr(Me)]AVP ($t=0.29$, NS) injected males. Treatment groups differed in time with the partner ($F(2,16)=4.09$, $P=0.04$) and time with the stranger ($F(2,16)=4.21$, $P=0.03$) due, in each case, to significant differences between the d$(CH_2)_5$[Tyr(Me)]AVP- and CSF-injected voles ($t_D=2.86$, $P<0.05$ in each case). Groups did not differ in activity as measured by number of entries into each of the chambers ($F(2,16)=0.54$, NS).

METHODS. Before exposure to sexually receptive females, sexually naive males were given a baseline intruder aggression test as described for Fig. 1. Each male then received a single i.c.v. injection of either artificial CSF (BioFluids, Inc., Rockville, MD; $n=13$); the OT antagonist OTA (Peninsula; $n=8$)[23]; or the V_{1a} antagonist d$(CH_2)_5$[Tyr(Me)]AVP (Peninsula; $n=6$–8 per dose)[24]. *Ex vivo* studies after central injection of d$(CH_2)_5$[Tyr(Me)]AVP (50 ng, i.c.v.) demonstrated persistent blockade of AVP-receptor binding for 18 h in the vole brain (data available on request), a time course which agrees with previous behavioural data in hamsters[25], our own behavioural data in voles (see Fig. 3*b*), as well as the time course of OTA activity[26]. Injections (2 µl) were given to halothane-anaesthetized voles using a 30-G needle attached via polyethylene-20 tubing to a 10 µl Hamilton syringe. Because the vole skull is lightly calcified, injections could be reliably given percutaneously by using a cranial band for placement over the lateral ventricle[27]. To verify placement, each injection included 10% india ink (v/v) and staining of the entire ventricular system was required at necropsy for inclusion of behavioural data (<10% rejected). After the injection, males were given sexually receptive females (ovariectomized and injected subcutaneously with 1.0 µg oestradiol benzoate for 2–4 days). Behaviour was recorded for the next 24 h with time-lapse videotaping. Females were then removed and males received a repeat intruder test. Aggression was scored as for Fig. 1. Reproductive behaviour scored from the time-lapse videotapes included latency to first mount and number of mounts. In an independent study, males ($n=19$) were similarly injected with CSF, OTA or d$(CH_2)_5$[Tyr(Me)]AVP just before mating, and about 24 h later they were assessed for partner preference as previously described[13] by measuring time in partner's (that is, mate's), stranger's (novel sexually receptive female) or neutral (uninhabited) cage over 3 h using time-lapse videotaping with 12:1 reduction. Partner preference was assessed by a paired *t*-test comparing time in partner's versus time in stranger's cage for each treatment, followed by a between-subject's factorial ANOVA to compare treatment groups on each measure.

behaviour 1 week later (Fig. 1). Attacks against the mate were not observed either during or after the initial 24-h mating experience, although females other than the partner were attacked as intruders (Fig. 1). Sexually naive males rarely show offensive aggression towards a conspecific female.

Mating seemed to be critical to the rapid induction of aggression, as males within 24 h of social but not sexual experience rarely show attack behaviour (Fig. 1). Mating has previously been shown in the female to help the development of a partner preference, critical for pair bonding in this species[13]. The development of aggression, essential for mate guarding and nest defence, may also be associated with pair bonding. In support of this hypothesis, no similar transition to aggression was seen in males of the closely related montane vole (*Microtus montanus*) which does not pair bond[14]. Montane males exhibited few attacks and threats when tested serially ($n=7$) before mating (9.7 ± 2.9), 24 h after mating (4.7 ± 1.6) or while living with a female as part of a breeding pair (5.8 ± 1.9).

To investigate a role for either AVP or OT in the induction of aggression, male prairie voles were injected intracerebroventricularly (i.c.v.) with either cerebrospinal fluid (CSF), the vasopressin-1a receptor (V_{1a}) antagonist [1-(β-mercapto-β,β-cyclopentamethylene propionic acid), 2-(O-methyl)tyrosine]-arginine-vasopressin (d$(CH_2)_5$[Tyr(Me)] AVP) or the OT receptor antagonist d$(CH_2)_5$-[Tyr(Me)$_2$, Thr^4, Tyr-NH$_2^9$]ornithine vasotocin (OTA) just before 24 h of exposure to a sexually receptive female. After either CSF or OTA injection, males showed mating-induced aggression (Fig. 1). Males injected with a wide range of V_{1a} antagonist doses failed to exhibit aggression after 24 h with a sexually receptive female (minimum effective dose 50.0 pg i.c.v.) (Fig. 2*a*). This failure could not be attributed to blockade of mating, as reproductive behaviour was unaffected by any dose of the antagonist (see Fig. 2 legend). Furthermore, the V_{1a} antagonist did not seem to be anti-aggressive *per se*. Breeder males with established selective aggression showed no decrease in attack or threat behaviour either 1 or 24 h after i.c.v. injection of the antagonist (5.0 ng) (Fig. 2*b*). Thus, AVP antagonism seemed to block the transition to aggression, not its expression.

To determine if the V_{1a} antagonist blocked the development of selective affiliation as well as aggression, males were tested for a partner preference after 24 h of mating. Injections and mating were as above, but after 24 h of mating each male was moved to the neutral cage of a three-chamber partner preference apparatus[13] with access to two adjoining cages, one with the mate (partner), the other with a novel female. Each female was loosely tethered to restrict movement out of her own cage. Males injected with CSF or OTA (5 ng, i.c.v.) before mating showed a significant preference for the mate 24 h later (Fig. 2*c*). After V_{1a} antagonist injection (5 ng, i.c.v.), males spent as much time with the stranger as the mate.

These results imply that physiological actions of endogenous AVP are essential for the induction of selective aggression and partner preference. But in initial studies in which we administered AVP (5–500 ng) as a single i.c.v. injection to sexually naive males, we observed no increase in aggression (data not shown). As the effects of sociosexual experience seemed to be mediated over several hours and the clearance of centrally injected AVP

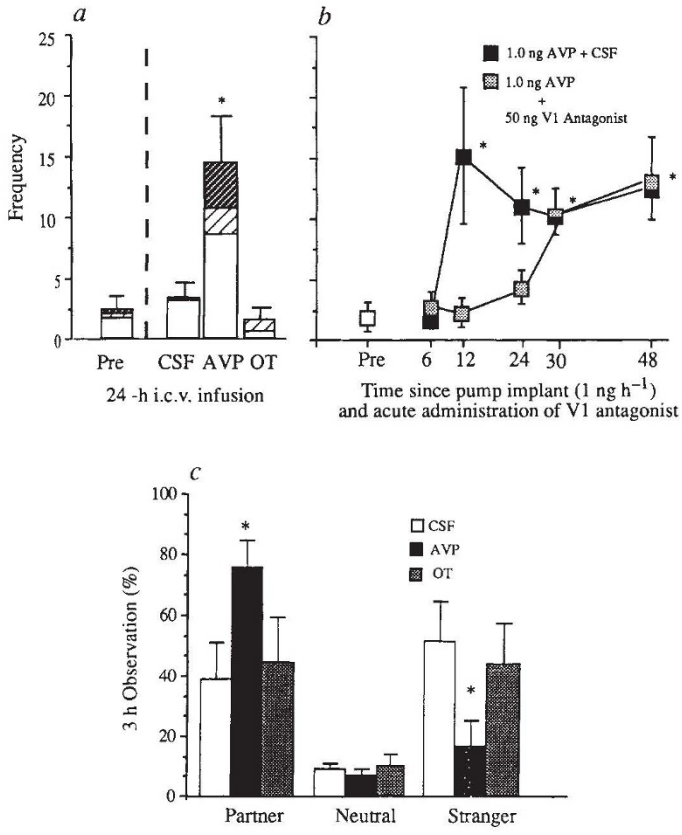

FIG. 3 Central infusion of AVP increases aggression and induces partner preference. *a*, After 24 h i.c.v. infusion of AVP (0.5 ng h^{-1}) but not CSF or OT (0.5 ng h^{-1}), aggression towards an intruder increases in male prairie voles. Groups did not differ before infusion (Pre), but in post-infusion resident–intruder tests, frequency of aggression was higher in AVP- than in either CSF- or OT-treated males ($F(2,22)=4.98$, $P=0.02$). (Fig. 1 legend; asterisk signifies $P<0.05$ comparison with CSF). *b*, In a separate study, aggression (attacks + threats) increased above baseline within 12 h of beginning AVP infusion (1.0 ng h^{-1}). Asterisk signifies significant ($P<0.05$) difference from baseline (Pre) aggression. Administration of a V_{1a} antagonist (50 ng, i.c.v.) at the start of infusion delayed the AVP-induced aggression by 18 h, yielding a temporal pattern significantly different from controls that received CSF i.c.v. instead of V_{1a} antagonist i.c.v. at the start of AVP infusion ($F(5,60)=3.06$, $P<0.05$). *c*, In a third group, during central infusion of CSF, OT or AVP (0.5 ng h^{-1}), each male was housed with a non-receptive female 'partner' for 24 h. In a subsequent preference test, CSF- and OT-treated males spent equal time with a novel female (stranger) and the partner ($t=0.51$, NS and 0.17, NS for CSF and OT, respectively). AVP-infused males spent more time with the partner than the stranger ($t=3.26$, $P=0.01$), with a significant overall treatment difference in time with the partner ($F(2,22)=3.3$, $P=0.05$) attributed to a difference between AVP- and CSF-treated males ($t_D=2.38$, $P=0.05$).

METHODS. AVP, OT or CSF was given to sexually naive males using a chronic i.c.v. infusion technique. Following a baseline resident–intruder test, voles were anaesthetized with chloropent (chloral hydrate (40 mg ml^{-1}) plus pentobarbital (10 mg ml^{-1})) (0.3 ml 100 g^{-1}) and stereotaxically fitted with a 25-G L-shaped cannula into the left lateral ventricle. The extra-cranial end of the cannula was fixed to the skull with dental cement and connected to PE20 tubing attached to an osmotic mini-pump (Alzet). The pump with attached tubing had been filled with CSF, OT or AVP (peptides from Peninsula) 18 h previously and primed by incubation in sterile saline at 37 °C. After recovery from anaesthesia, males were returned to their home cages and tested for aggression towards an intruder at various times thereafter. In an initial study (*a*), AVP ($n=9$), OT ($n=8$) or CSF ($n=8$) was administered via mini-pump and aggression was assessed 24 h later. In a second study (*b*), either CSF ($n=6$) or d$(CH_2)_5$[Tyr(Me)]AVP (50 ng, $n=6$) was administered through the cannula at the time of implantation to determine the time course and specificity of AVP effects. In a third study (*c*), males ($n=25$) were implanted with pumps, housed with females for 24 h and then assessed for partner preference as described for Fig. 2.

has been reported to be within minutes[15], a single injection of agonist may be insufficient to mimic physiological changes in AVP. To achieve prolonged elevated brain concentrations of peptide, we administered AVP (and OT) i.c.v. by osmotic mini-pump for 48 h to voles receiving neither sexual nor social experience. Males became aggressive after AVP (0.5 ng h^{-1}) but not after OT (0.5 ng h^{-1}) or CSF infusion (Fig. 3*a*). This dose of AVP increased AVP concentrations in the septum threefold (0.94 ± 0.29 pg per mg tissue versus 0.31 ± 0.10 pg per mg tissue comparing AVP and CSF group; $n=9$) using a standard radioimmunoassay (kit from Peninsula) with microdissected tissue after a 24-h infusion. Aggression appeared within 12 h of starting AVP administration and seemed to be mediated via a V_{1a} receptor, as prior injection with the V_{1a} antagonist delayed the initiation of aggression by 18 h (Fig. 3*b*). The aggressive behaviour after AVP seemed to be qualitatively similar to that seen post-mating, although the intensity of attacks was slightly less with exogenous peptide, possibly due to the encumbrance of the mini-pump. Nevertheless, the data demonstrate that activation of the V_{1a} receptor is both necessary and sufficient for the development of selective aggression in the male prairie vole.

To determine if exogenous AVP also induced a partner preference, a separate group of males received i.c.v. infusion of AVP, OT or CSF by mini-pump while being housed with an ovariectomized (that is, non-receptive) female for 24 h. In a 3-h choice test, CSF- and OT-injected males showed no preference (spent roughly equal times in partner's cage and that of the novel female). Males injected with AVP (0.5 ng h^{-1}) spent 75.4% of the available time with the partner and 16.7% with the stranger (Fig. 3*c*). The groups did not differ on number of entries into the two cages ($F(2,22)=0.40$, not significant (NS)), suggesting that activity level was not affected by AVP.

Our results, implicating AVP in the development of selective aggression and partner preference, are consistent with recent data suggesting an important role for AVP in mediating parental behaviour in prairie voles[8]. Thus, it seems that AVP may be integral to several of the behaviours characterizing monogamous social organization in general and the process of pair bonding in particular. Studies in non-monogamous mammals have implicated AVP in social memory in rats[10], territorial marking in hamsters[9] and aggression in mice[16,17]. There are also extensive data demonstrating AVP effects on memory and learning, thermoregulation, and grooming—effects with only an indirect relationship to affiliative behaviours, particularly in commonly used research animals such as rats and mice which do not pair bond (see, for instance, ref. 18). In a species that forms long-term selective bonds, however, memory of a mate, huddling for thermal comfort and grooming are important aspects of affiliation.

Our results are consistent with the hypothesis that the activation of central AVP pathways by mating is an essential neural correlate of pair bonding in the male prairie vole. Oxytocin and not AVP increases in CSF after ejaculation in the rat, a polygynous rodent[19], whereas in male prairie voles AVP is apparently released after mating[20]. AVP secretion has previously been described with sexual arousal in human males[21], but the extent to which any single peptide subserves any aspect of social bonding in humans remains entirely speculative. □

Received 1 April; accepted 8 July 1993.

1. Kleiman, D. G. *Q. Rev. Biol.* **52,** 39–69 (1977).
2. Clutton-Brock, T. H. *Proc. R. Soc. Lond.* **236,** 339–372 (1989).
3. Dewsbury, D. A. in *American Zoology Nebraska Symposium on Motivation* (ed. Leger, D. W.) 1–50 (Univ. Nebraska Press, Lincoln, 1988).
4. Getz, L. L., Carter, C. S. & Gavish, L. *Behav. Ecol. Sociobiol.* **8,** 189–194 (1981).
5. Carter, C. S., Getz, L. L. & Cohen-Parsons, M. in *Advances in the Study of Behavior* (eds Rosenblatt, J. S., Beer, C., Busnel, M. C. & Slater, P. J. B.) 109–145 (Academic, New York, 1986).
6. Insel, T. & Shapiro, L. *Proc. natn. Acad. Sci. U.S.A.* **89,** 5981–5985 (1992).
7. Insel, T. R. *et al. Regul. Peptides* **45,** 127–131 (1993).
8. Bamshad, M., Novak, M. & deVries, G. *J. Neuroendocr.* **5,** 247–256 (1993).
9. Ferris, C. F., Albers, H. E., Wesolowski, S. M. & Goldman, B. D. *Science* **224,** 521–523 (1984).
10. Dantzer, R., Bluthe, R.-M., Koob, G. F. & Le Moal, M. *Psychopharmacology* **91,** 363–368 (1987).

11. Insel, T. R. *Psychoneuroendocrinology* **17,** 3–33 (1992).
12. Miczek, K. A. & Winslow, J. T. in *Experimental Psychopharmacology* (eds Greenshaw, A. J. & Dourish, C. T.) 27–113 (Humana, Clifton, New Jersey, 1987).
13. Williams, J. R., Catania, K. C. & Carter, C. S. *Horm. Behav.* **26,** 339 (1992).
14. Jannett, F. J. *Biologist* **62,** 3–19 (1980).
15. Jones, P. M. & Robinson, I. C. A. F. *Neuroendocrinology* **34,** 297–302 (1982).
16. Roche, K. E. & Leshner, A. I. *Science* **204,** 1343–1344 (1979).
17. Compaan, J. C., Buijs, R. M., Pool, C. W., de Ruiter, A. J. H. & Koolhaas, J. M. *Br. Res. Bull.* **30,** 1–6 (1993).
18. De Wied, D., Elands, J. & Kovaks, G. *Proc. natn. Acad. Sci. U.S.A.* **88,** 1494–1498 (1991).
19. Hughes, A. M., Everitt, B. J., Lightman, S. L. & Todd, K. *Br. Res.* **414,** 133–137 (1987).
20. Bamshad, M., Novak, M. & deVries, G. *Soc. Neurosci. Abstr.* **18,** 152.10 (Soc. for Neuroscience, Anaheim, California, 1992).
21. Murphy, M. R., Seckl, J. R., Burton, S., Checkley, S. A. & Lightman, S. L. *J. clin. Endocr. Metab.* **65,** 738–741 (1987).
22. Pierce, J. D. J., Pellis, V. C., Dewsbury, D. A. & Pellis, S. M. *Aggressive Behav.* **17,** 337–349 (1991).
23. Elands, J. *et al. Eur. J. Pharmac.* **147,** 197–207 (1987).
24. Kruszynski, M. *et al. J. med. Chem.* **23,** 364 (1980).
25. Ferris, C. F., Singer, E. A., Meenan, D. M. & Albers, H. E. *Eur. J. Pharmac.* **154,** 153–159 (1988).
26. Witt, D. M. & Insel, T. R. *Endocrinology* **128,** 3269–3276 (1991).
27. Popick, F. R. *Life Sci.* **18,** 197–204 (1975).

African and North American populations of *Drosophila melanogaster* are very different at the DNA level

David J. Begun & Charles F. Aquadro

Cornell University, Section of Genetics and Development, Biotechnology Building, Ithaca, New York 14853, USA

UNDERSTANDING **genetic evolution within species requires an accurate description of variation within and between populations and the ability to distinguish between the potential causes of an observed distribution of variation. In the cosmopolitan species *Drosophila melanogaster*, previous studies suggested that gene flow within and between continents is extensive[1] and that most of the nuclear gene variation is found within, rather than among, populations[2,3]. Here we present evidence that a population from Zimbabwe is more than twice as variable as those from the United States of America at the DNA sequence level, that most variants are not shared between the two geographic regions, and that there are nearly fixed differences between the Zimbabwe and USA samples in genomic regions experiencing low recombination rates. It appears that there is an unappreciated degree of population structure in *D. melanogaster* and that equilibrium models of molecular evolution are inappropriate for this species.**

In Table 1 we present our four-cutter restriction site polymorphism data from seven X-linked gene regions in *D. melanogaster* chromosomes sampled from Zimbabwe; we also show our data and previously published data from the same genes in samples from the USA[4–7]. With the exception of *ac*, each gene is more variable in Zimbabwe than in the USA. Overall, there are more than twice as many segregating sites in Zimbabwe (167) as in the USA (77). The difference in proportion of segregating sites in the two samples is highly significant (Fisher's exact test, $P<0.0001$; this test is appropriate because the sample sizes for Zimbabwe and the USA are roughly equal) and the populations are significantly differentiated ($P<0.001$) at each of the seven genes examined[8]. Only 44 of a total of 200 polymorphic restriction sites (22%) scorable in both Zimbabwe and the USA are observed in both geographic regions. This difference is not attributable solely to rare 'private' alleles. For example, in the USA the mean frequencies of *white* and *G-6pd* locus variants present in the USA but absent from Zimbabwe are 0.16 (±0.042) and 0.23 (±0.058), respectively. The contrasting Tajima D values[9] (Table 1) for the two samples (especially in regions of 'normal' crossing-over) indicate that there is a tendency for Zimbabwe to harbour more low-frequency variants than the USA, providing further evidence of different evolutionary processes in these populations. But the difference in variability is not simply a consequence of the presence of rare alleles in Zimbabwe which are absent in the USA. Even for estimates of nucleotide diversity, π, which are only weakly affected by rare variants, the Zimbabwe sample is two- to sixfold more heterozygous for most loci.

Our results contrast with previous comparisons of nuclear gene variability in African and USA samples of *D. melanogaster*. Restriction site data suggested that the *y-ac-sc* and *G-6pd* loci were slightly more variable in Botswana than in the USA; however, the high levels of linkage disequilibrium and small number of bases surveyed made it difficult to assess the significance of the observation[10,11]. Levels of allozyme variation in Benin and USA samples of *D. melanogaster* (Table 2) were virtually identical and 57 of 84 allozyme variants (68%) were shared between geographic regions[2,12]. The difference in the proportion of shared variants in our restriction site data versus the allozyme data is highly significant (Fisher's exact test, $P<0.0001$; this test is conservative given the different sample sizes for the USA and Africa allozyme data). Thus, our four-cutter data are the first good evidence that there is a vast reservoir of previously unknown nuclear DNA polymorphism segregating in Africa.

Although it is thought that *D. melanogaster* left Africa to colonize temperate regions within the past several thousand years[13], earlier data did not support the hypothesis that a wholesale loss of nuclear gene variability accompanied the colonization. Our data, however, are consistent with the notion that a considerable loss of variation occurred in some populations during the history of the *D. melanogaster* lineage. The data are compatible with several hypotheses, including a partial bottleneck in the lineage(s) from which USA populations are derived and the notion that only a subset of 'ancestral' types were selectively favoured to leave Africa during the colonization process. Whatever the case, it is no longer realistic to view *D. melanogaster* populations as being near equilibrium.

Earlier interpretations of allozyme data from *D. melanogaster* attributed the few frequency differences between populations to selection on allozyme variants prevailing over extensive gene flow[1,2]. Because a large majority of observed polymorphic restriction sites in our data are located in flanking or intronic sequences, most of the differences between Zimbabwe and the USA may be explained in terms of mutation and drift rather than selection. Furthermore, our results cannot be explained solely by differences in the frequency of protein electrophoretic variants between populations. For example, even within a single electromorph class at *Pgd* (Fast), the Zimbabwe (sample size $n=49$) and USA ($n=32$) samples shared no four-cutter haplotypes and only 7 of 28 polymorphic sites (data not shown). There are at least two (not mutually exclusive) potential explanations for the contrast between the DNA data (Table 1) and the allozyme data (Table 2). First, there may be significant population structure within Africa; we note that our Zimbabwe sample has not been surveyed for allozyme variation. Second, we cannot rule out the possibility that restriction site and allozyme variation are experiencing substantially different evolutionary dynamics (such as selection on allozyme variation).

The Zimbabwe four-cutter data provide novel and qualitatively different inferences about selection from those possible from allozyme frequencies. As had been seen in a heterogeneous sample[14], nucleotide heterozygosity is positively correlated with regional rates of recombination (Fig. 1). Hitch-hiking effects of advantageous or deleterious alleles have been proposed to contribute to this pattern[14–17]. In particular, the *ac* and *su(f)* regions appear to have significantly less variation than expected under a neutral model (Table 2 legend). Although several shared polymorphic sites in Zimbabwe and the USA are found at different frequencies in the two samples, the most extreme cases