

Balancing selection maintains polymorphisms at neurogenetic loci in field experiments

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Most variation in behavior has a genetic basis, but the processes determining the level of diversity at behavioral loci are largely unknown for natural populations. Expression of arginine vasopressin receptor 1a (*Avpr1a*) and oxytocin receptor (*Oxtr*) in specific regions of the brain regulates diverse social and reproductive behaviors in mammals, including humans. That these genes have important fitness consequences and that natural populations contain extensive diversity at these loci implies the action of balancing selection. In *Myodes glareolus*, *Avpr1a* and *Oxtr* each contain a polymorphic microsatellite locus located in their 5' regulatory region (the regulatory region-associated microsatellite, RRAM) that likely regulates gene expression. To test the hypothesis that balancing selection maintains diversity at behavioral loci, we released artificially bred females and males with different RRAM allele lengths into field enclosures that differed in population density. The length of *Avpr1a* and *Oxtr* RRAMs was associated with reproductive success, but population density and the sex interacted to determine the optimal genotype. In general, longer *Avpr1a* RRAMs were more beneficial for males, and shorter RRAMs were more beneficial for females; the opposite was true for *Oxtr* RRAMs. Moreover, *Avpr1a* RRAM allele length is correlated with the reproductive success of the sexes during different phases of reproduction; for males, RRAM length correlated with the numbers of newborn offspring, but for females selection was evident on the number of weaned offspring. This report of density-dependence and sexual antagonism acting on loci within the arginine vasopressin–oxytocin pathway explains how genetic diversity at *Avpr1a* and *Oxtr* could be maintained in natural populations.

Avpr1a | *Oxtr* | sexual conflict | density-dependent selection | *Myodes glareolus*

Most variation in behavior has a substantial genetic basis. Identifying loci that underpin the expression of behavior is central to our understanding of the evolution and adaptive significance of behavioral diversity (1, 2). Although many studies have found an association between genotype and behavior (2–4), few have quantified the eco-evolutionary dynamics of these genetic polymorphisms. A corollary of the diversity of behaviors exhibited in wild populations is the action of balancing selection (3, 5), a general term for mechanisms that promote fitness of alternate genotypes, including density-dependent selection (1), negative frequency-dependent selection (6), heterozygote advantage (7), and sexual antagonism (8, 9). Density- and frequency-dependent selection, for example, can maintain polymorphisms at the foraging gene in laboratory populations of *Drosophila melanogaster* (1, 10). However, the lack of evidence for the conditions that drive balancing selection on behavioral loci in natural settings creates a challenge to behavioral genetics in understanding the dynamics of behavioral loci in real-world scenarios. Genes within the arginine vasopressin–oxytocin pathway present a classic opportunity to meet this challenge; its constituent loci have been subject to extensive study because they exert major effects on animal behavior (5, 11, 12).

The neurotransmitters vasopressin and oxytocin are evolutionarily conserved, with the vasopressin–oxytocin pathway regulating social and reproductive behaviors in many mammals including humans (5, 11, 13, 14). The behaviors associated with vasopressin

and oxytocin are often mediated by the density of their receptors, notably arginine vasopressin receptor 1a (V1aR) and oxytocin receptor (OTR), in specific regions of the brain (5, 11–13). The genetic basis of the variation in V1aR density and its concomitant effect on behavior has been studied comprehensively in microtine voles (5, 15–17). In the prairie vole *Microtus ochrogaster*, arginine vasopressin receptor 1a (*Avpr1a*) expression and V1aR density in specific regions of the brain correlate with allele length at a regulatory region-associated microsatellite (RRAM) located in the 5' regulatory region of the *Avpr1a* gene (15, 16), and longer *Avpr1a* RRAM alleles are associated with greater partner preference and male parental care in the laboratory (15). This intraspecific pattern of an association between *Avpr1a* RRAM allele length and V1aR expression in the brain and/or socio-reproductive behavior extends to other mammals. In chimpanzees, genetic diversity at one *Avpr1a* RRAM locus is associated with sociality (18). In humans, allele length at the *Avpr1a* RRAM locus RS3 is correlated with gene expression in the hippocampus (19) and with male pair bonding (14), altruism (19), and maternal behavior (20), whereas allele length at a second *Avpr1a* RRAM locus (RS1) correlates with autism and promoter activity (21). An association between RRAM allele length and transcriptional activity is not unique to *Avpr1a* and has been shown in other genes and in diverse taxa (22, 23).

In contrast to *Avpr1a*, no genetic polymorphism in the 5' regulatory region of the oxytocin receptor (*Oxtr*) that associates with variation in OTR density in the brain has been identified. Nonetheless, the region ~1–5 kbp upstream of *Oxtr* is important for the

Significance

Arginine vasopressin receptor 1a (*Avpr1a*) and oxytocin receptor (*Oxtr*) are evolutionarily conserved loci that affect socio-reproductive behavior in many animals. That these loci affect fitness and exhibit substantial genetic variation in wild populations raises questions about the processes that maintain genetic variation at these loci. We show that the length of microsatellites located in the 5' regulatory regions of *Avpr1a* and *Oxtr* are associated with reproductive success and gene expression in the brain. Crucially, balancing selection through sexually antagonistic fitness effects and density-related social influences is capable of maintaining microsatellite length polymorphisms at both genes. The action of sex and population density operating at two loci indicates that balancing selection may maintain diversity at many other behavioral loci.

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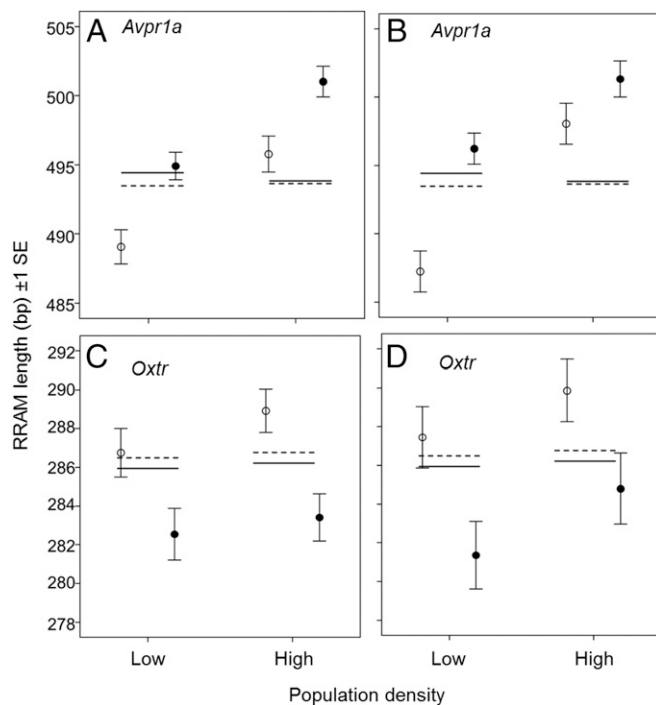


Fig. 2. Sex-specific and density-dependent selection of *Avpr1a* and *OxtR* loci in the field. Mean lengths (bp $\pm$ 1 SE) of the paternally (black circles) and maternally (white circles) derived alleles at *Avpr1a* (A and B) and *OxtR* (C and D) RRAM loci in bank vole offspring produced at high and low population densities. Newborn offspring are shown in A and C; recruited offspring are shown in B and D. Reproductively successful males had significantly longer *Avpr1a* alleles than females, whereas the opposite pattern was observed for *OxtR* allele lengths. Furthermore, increasing population density selected for longer *Avpr1a* alleles in both sexes. The mean *Avpr1a* (A and B) and *OxtR* (C and D) RRAM allele lengths in the parental generation are shown as solid (males) and dashed (females) lines.

density. Male reproductive success also showed an interaction of population density $\times$ *Avpr1a* RRAM allele length, but with the main effect operating on the number of sired offspring (newborn animals) ($P = 0.011$) (Table 1 and Table S3), not the number of recruited offspring ($P = 0.130$) (Table 1 and Table S3). At high density, an ~ 30 bp increase in *Avpr1a* RRAM allele length in males corresponded to the production of one more newborn offspring, but *Avpr1a* genotype had no apparent fitness effect at low density. *OxtR* had a significant impact only on male reproductive success at low population density; males with shorter alleles sired more offspring ($P < 0.001$ for allele length; $P = 0.011$ for allele length $\times$ density) (Fig. 3D, Table 1, and Table S3) and achieved more recruited offspring ($P < 0.001$ for allele length; $P = 0.002$ for allele length $\times$ density) (Fig. 3E, Table 1, and Table S3). In effect, reducing a male *OxtR* genotype by about 20 bp corresponds to an increase in fitness of one additional recruited offspring at the low population density.

Additional evidence for sex-specific optima was apparent by quantifying the lengths of the maternal and paternal alleles in the offspring (Fig. 2). There was a trend to produce offspring with longer RRAM alleles at a high population density (Fig. S3), although this density effect was significant only for *Avpr1a* (Table S4). At both high and low population densities, offspring inherited significantly longer *Avpr1a* alleles from males than from females ($P < 0.001$ for origin of allele) (Fig. 2A and B and Table S4). At *OxtR*, we found the opposite pattern, with offspring inheriting significantly longer RRAM alleles from their mothers than from their fathers ($P = 0.004$ for origin of allele) (Fig. 2C and D and Table S4). These results indicate sex-specific fitness optima for both *Avpr1a* and *OxtR* alleles.

Discussion

Genes within the arginine vasopressin–oxytocin pathway provide some of the best-studied models of the link from gene to brain to socio-sexual behavior (5, 11–14), but the mechanisms that can maintain high phenotypic and genetic variation in these loci are not known (5). Our field experiments show how RRAM genotypes at both *Avpr1a* and *OxtR* affect reproductive success, in agreement with some work on the *Avpr1a* RRAM in the prairie vole (33, 34), and provide insight into the dynamics of the *OxtR* locus. The major advance in understanding the eco-evolutionary dynamics of the arginine vasopressin–oxytocin pathway is that both loci have sex- and population density-specific fitness optima. Genetic diversity at these loci thus has adaptive relevance in natural settings and is likely maintained by balancing selection.

That sex and population density interact to vary the fitness optima for alleles at *Avpr1a* and *OxtR* RRAM loci provides plausible mechanisms for the maintenance of genetic diversity at these loci (33, 34). Apparent functional divergence between sexes can maintain polymorphisms by generating different optimal trait values between the sexes via sexual antagonism (8, 9). A taxonomically widespread influence of sexually antagonistic alleles is supported by empirical studies on quantitative traits (e.g., testosterone level, body size) (6, 43) and at specific loci (44–46). Some authors have argued that sexual antagonism alone may be insufficient to account for most natural patterns of genetic diversity (6) but that instead some interaction with changes in social environment, such as fluctuation in population density, is required (6, 42).

Changes in population density (40, 41) can impact components of fitness through intraspecific competition (e.g., for food, mates, and territories). Competitive interactions for resources are often resolved by an individual's level of aggression, a behavior regulated by *Avpr1a* and *OxtR* (13, 47). Interestingly, male prairie voles with divergent *Avpr1a* genotypes enjoy similar overall fitness that is achieved via different mechanisms, being associated with either an apparent capability to monopolize a female partner or increased extra-pair fertilization (5). Okhovat et al. (5) suggested that population density could dictate the strength and direction of selection acting on divergent *Avpr1a* genotypes, with population density cycles thus maintaining genetic diversity. Therefore it is relevant that we observed an interaction between population density and *Avpr1a* RRAM allele length in bank voles, in contrast to a field study on prairie voles in which males with shorter *Avpr1a* RRAM alleles enjoyed greater reproductive success irrespective of density treatment (33), likely a response to the greater competition at high population density. More generally, high population density selects for longer alleles at both loci and in both sexes of the bank vole (Fig. 2). By analogy, these results imply selection for increased gene expression (15, 17, 19, 21), raising the possibility that the optimum female *Avpr1a* genotype at high population density represents a shift toward the male optimum genotype, and the male allelic optimum for *OxtR* at high population density represents a shift toward the female optimum (Fig. 2). Conversely, there is the possibility of sex-specific gene expression associated with genotype (e.g., *Avpr1a* in the midbrain) (Fig. 1D) and for still further fine-scale variation in V1aR and OTR receptor density in the brain (12, 15). Indeed, no association between genotype and behavior was identified in female prairie voles at *Avpr1a* (15). Processes such as the activation of hormone receptors can drive sex-specific gene expression; for example, estrogen receptor mediates the transcriptional activity of many genes, including the expression of *OxtR* (48). Nonetheless, examining several processes in tandem demonstrates how intralocus sexual conflict can be dynamic through an interrelationship with the social environment (6, 42). Interactions between different mechanisms of balancing selection have a fundamental role in maintaining diversity.

Intraspecific interactions determine reproductive success, but the severity and timing of competition often differ between sexes (6, 35,

analyzed using population density and origin of allele (maternal or paternal RRAM allele) and their interactions as independent variables. Offspring ID nested within litter and experimental enclosure was included as a random effect. We used linear models with R v.3.1.1 (R Development Core Team 2014) to analyze the effects of allele length, sex, brain region, and their interactions on the expression of *Avpr1a* and *Oxtr* (Table S1). *Avpr1a* and *Oxtr* expression also was analyzed separately for each brain region (Table S2).

Ethical Approval. Use of study animals followed the ethical guidelines for animal research in Finland.

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