Supplementary Discussion

Differential demand of care by *P. polionotus* and *P. maniculatus* pups

Differences in parental care may occur because *P. polionotus* are inherently more parental or because their pups demand more care than *P. maniculatus* pups. To distinguish between these possibilities, we compared the parental behaviour of animals towards their own pups to the behaviour towards pups from the other species. We found that *P. polionotus* fathers licked *P. maniculatus* pups more than their own pups, and that *P. maniculatus* mothers licked and huddled *P. polionotus* pups less than their own pups, both of which oppose the hypothesis that *P. polionotus* pups demand more care. Apart from these cases, fathers and mothers of both species behaved indistinguishably towards their own pups and pups of the other species (Extended Data Fig. 2), indicating that the higher levels of parental behaviour in *P. polionotus* are not due simply to an increased demand of care by their pups.

Genetic architecture of parental behaviour

We identified 12 non-overlapping QTLs on 11 chromosomes: the 11 QTLs shown in Fig. 4b plus another QTL on chromosme 11 associated with licking in females (Extended Data. Fig. 3b). With an increased sample size, some of these QTLs may fractionate into more independent QTLs. For example, chromosome 1 has a QTL that affects both nest quality and licking behaviour; their peaks are far from each other but the QTL support intervals overlap (Fig. 4b). However, six of the 12 QTL peaks match between the independent scans in the two sexes or across behaviours, a consistency that

suggests these QTLs are true positives. Additional experiments are necessary to confirm that other QTLs are not false positives.

Overall, we found 23 associations between QTLs and specific behaviours in each sex (Extended Data Fig. 4). In males, the *P. polionotus* allele was associated with "higher" parental care in nine of these 23 cases, with "lower" parental care in eight cases, heterozygous animals showed higher care in one case, and the QTL explained only a small amount of the behavioural variance (i.e., it explains less than 1% of the variance among the F2s) in five cases. In females, the *P. polionotus* allele was associated with "higher" parental care in five of 23 cases, with "lower" parental care in eight cases, heterozygous animals showed higher care in five cases, and the QTL had a low explanatory power for behavioural variation in five cases. Interestingly, in five out of 23 QTL-behaviour associations, the *P. polionotus* allele was associated with more parental care in fathers but less in females and in two QTL-behaviour associations it was the *P. maniculatus* allele that increased parental care in fathers and decreased it in mothers. These patterns suggest an absence of directional selection for increased parental care in monogamous *P. polionotus*; that complex epistatic interactions confound these results (if, for example, the *P. polionotus* alleles that decrease parental care actually increase it in a *P. polionotus* genetic background); or that balancing selection acting on sexually dimorphic QTL prevent directional selection because an allele can promote maternal care but suppress paternal care, or *vice versa*.

Relationship between anxiety and parental behaviour

To determine the extent to which interspecific differences in anxiety contribute to variation in parental behaviour, we recorded the time animals spent in the open arms of an elevated plus maze, a test widely used to measure anxiety in rodents[1]. Overall, *P. maniculatus* spent less time in the open arms compared with *P. polionotus*, indicating *P. maniculatus* displays more anxiety-like behaviour (Extended Data Fig. 9a). However, we found only weak correlations between anxiety-related behaviour and parental behaviours (r_s 0.001-0.15), with the highest correlation between time in open arms and promptness to approach pups by males (Extended Data Fig. 9b). Moreover, the peak lod score on chromosome 4 for time in open arms is far from the nest-building QTL on that chromosome (Extended Data Fig. 9c). Finally, vasopressin administration to *P. polionotus* affected nest-building but not other aspects of parental care that we may also expect to be similarly affected by changes in anxiety (Fig. 5c). Together, our results indicate that, while vasopressin is known to promote anxiety in rodents[2], variation in vasopressin levels affects parental nest building independent of differences in anxiety in these two species.

Chemogenetics of vasopressin neurons in *Mus musculus*

To support our finding that vasopressin inhibits nest building and to determine if more targeted vasopressin manipulations can alter nest building, we performed chemogenetic experiments to excite or inhibit specific vasopressin neurons in the hypothalamus. Because several nuclei of the hypothalamus and the bed nucleus of the stria terminalis show similar levels of allele-specific expression of vasopressin transcripts

(Extended Data Fig. 8c), we decided to manipulate the activity of the vasopressin neurons in the PVN, since this nucleus has been implicated previously in nest-building behaviour by lesion studies[3]. First, we generated *Avp-Cre* transgenic *Mus musculus* mice to selectively target vasopressin neurons (Methods and Extended Data Fig. 10a). Then, we injected a virus containing either an inhibitory or an excitatory Cre-dependent synthetic receptor (known as DREADD[4]) into the PVN of the *Avp-Cre* transgenic mice (Extended Data Fig. 10b); these receptors modulate neuronal activity upon activation by intraperitoneal injection of the synthetic ligand Clozapine N-oxide (CNO). Consistent with the negative relationship between vasopressin and nest-building behaviour, inhibiting vasopressin neurons increased nest building in males an average of 2.6-fold (P=0.002; Extended Data Fig. 10c), whereas activating these neurons led to a 2.4-fold decrease in nest building in females P=0.001; Extended Data Fig. 10d). Together, these results indicate that PVN vasopressin neurons inhibit nest building in mice and further support the notion that variation in vasopressin levels contributes to differences in parental nest building in *Peromyscus*.

The observation that inhibiting vasopressin neurons promotes nest building only in males and activating the neurons suppresses nest building only in females suggests that male *Mus musculus* are in a high vasopressin state (that cannot be further increased), while females are in a low vasopressin state (that cannot be furthered decreased). This model is consistent with the known sexual dimorphism in the vasopressin system of many mammals (including humans) and other vertebrates, in which vasopressin signaling is consistently more prominent in males[5–8]. Interestingly, vasopressin mRNA in the *Peromyscus* hypothalamus does not differ between sexes (Extended Data Fig. 7a), the

QTL that encompasses the vasopressin gene affects nest building in both males and females (Fig. 4b), and vasopressin administration reduced nest building of both male and female *P. polionotus* (Fig. 5c). This indicates that the effect of vasopressin on nest-building in *P. polionotus* is not sexually dimorphic as it is in *M. musculus*.

Supplementary References

1. Walf, A. A. & Frye, C. A. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat. Protoc.* **2,** 322–28 (2007).
2. Neumann, I. D. & Landgraf, R. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. *Trends Neurosci.* **35,** 649–59 (2012).
3. Insel, T. R. & Harbaugh, C. R. Lesions of the hypothalamic paraventricular nucleus disrupt the initiation of maternal behavior. *Physiol. Behav.* **45,** 1033–41 (1989).
4. Krashes, M. J. *et al.* Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *J. Clin. Invest.* **121,** 1424–8 (2011).
5. Ishunina, T. A. & Swaab, D. F. Vasopressin and oxytocin neurons of the human supraoptic and paraventricular nucleus: size changes in relation to age and sex. *J. Clin. Endocrinol. Metab.* **84,** 4637–44 (1999).
6. de Vries, G. J. & Panzica, G. C. Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: different mechanisms, similar endpoints. *Neuroscience* **138,** 947–55 (2006).
7. Kelly, A. M. & Goodson, J. L. Functional significance of a phylogenetically

widespread sexual dimorphism in vasotocin/vasopressin production. *Horm. Behav.* **64,** 840–6 (2013).

8. Delville, Y., Koh, E. T. & Ferris, C. F. Sexual differences in the magnocellular vasopressinergic system in golden hamsters. *Brain Res. Bull.* **33,** 535–540 (1994).