population mean heterozygosity (i.e. 12 data points). Both analyses were statistically significant, indicating that populations with low heterozygosity have relatively abnormal sperm.

Two alternatives to inbreeding depression are consistent with this result: a third, unknown factor causes some populations to have low heterozygosity and relatively abnormal sperm; for instance, environmental heterogeneity or genetic drift could be such factors. Alternatively, populations with relatively abnormal sperm could show high rates of infertility and decline in size, thereby causing heterozygosity to decline; i.e., sperm abnormality affects heterozygosity rather than vice versa.

Convincing evidence of inbreeding depression requires a significant relationship between heterozygosity and sperm abnormalities across individuals within a population. Most of Gage *et al.*'s [1] population samples were too small to detect inbreeding depression. There is no relationship between heterozygosity and sperm quality within the largest population (n = 29), although a significant relationship was detected in another population (n = 13). Furthermore, heterozygosity of an individual can be a poor indicator of inbreeding coefficient [4–6].

A recently proposed method [4] was used by Gage *et al*. [1] to test whether marker heterozygosity reflects the inbreeding coefficient, but it was applied simultaneously to all populations. The relevant question of whether markers can be used as a proxy for the inbreeding coefficient within a population is not addressed.

There are several methods to test for inbreeding depression when discrete populations are sampled. If sample sizes permit — Gage *et al*.'s do not — analyses could be conducted within each population separately or, alternatively, 'population' could be fitted as a categorical term in a model that includes all individuals. There is also a large literature on more refined methods to control for population structure in genetic association studies (reviewed in [7]).

We have raised the issue of population structure because we have noticed a new trend in heterozygosity–fitness correlation studies, namely a failure to deal with population structure, e.g. [8]. In general, evolutionary geneticists should consider population stratification as the most likely cause of associations between genetic markers and a focal trait and try to exclude this explanation before testing other explanations.

References

1. Gage, M.J.G., Surridge, A.K., Tomkins, J.L., Green, E., Wiskin, L., Bell, D.J., and Hewitt, G.M. (2006). Reduced heterozygosity depresses sperm quality in wild rabbits, *Oryctolagus cuniculus*. Curr. Biol. *16*, 612–617.
2. Surridge, A.K., Bell, D.J., and Hewitt, G.M. (1999). From population structure to individual behaviour: genetic analysis of social structure in the European wild rabbit (*Oryctolagus cuniculus*). Biol. J. Linn. Soc. *68*, 57–71.
3. Lynch, M., and Walsh, B. (1998). Genetics and Analysis of Quantitative Traits (Sunderland, Massachusetts: Sinauer).
4. Balloux, F., Amos, W., and Coulson, T. (2004). Does heterozygosity estimate inbreeding in real populations? Mol. Ecol. *13*, 3021–3031.
5. Pemberton, J. (2004). Measuring inbreeding depression in the wild: the old ways are the best. Trends Ecol. Evol. *19*, 613–615.
6. Slate, J., David, P., Dodds, K.G., Veenvliet, B.A., Broad, T.E., and McEwan, J.C. (2004). Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. Heredity *93*, 255–265.
7. Ewens, W.J., and Spielman, R.S. (2001). Locating genes by linkage and association. Theoret. Pop. Biol. *60*, 135–139.
8. Acevedo-Whitehouse, K., Vicente, J., Gortazar, C., Hofle, U., Fernandez-De-Mera, I.G., and Amos, W. (2005). Genetic resistance to bovine tuberculosis in the Iberian wild boar. Mol. Ecol. *14*, 3209–3217.

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Response to Slate and Pemberton

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In their correspondence, Slate and Pemberton [1] argue that the key interpretation of our study, namely the finding of a negative correlation between heterozygosity and sperm abnormality across wild UK rabbits [2], is flawed. According to their view, the reason for this is that our analysis did not adequately deal with population stratification. Slate and Pemberton [1] claim that demonstrations of heterozygosity–fitness correlations are only convincing when heterozygosity–fitness relationships are found across individuals within populations.

Slate and Pemberton [1] rightly point out that sampling individuals from different geographic origins could confound associations because of environmental heterogeneity at the sites. Such problems are inherent to all correlative studies in the natural environment. There is a subjective problem of defining which level of a 'population' will actually control for environmental heterogeneity. In our system, for instance, mobile male rabbits [3] will be exposed to uncontrolled environmental variation even within a 'site'.

In our study [2], we explore some potential environmental heterogeneity: the correlation of population heterozygosity means shows that the relationship holds; moreover, there is no covariance with body mass or condition, which are possible indicators of environmental influence. The relationship we found also holds across separate 'mainland' or 'island' samples.

Moreover, we applied the model suggested by Slate and Pemberton [1] and controlled for 'population' by fitting it as a categorical term. Across individuals, the relationship remains significant ($F = 23.3$, $P < 0.0001$) (similar

if we also include body mass, $F = 15.8$, $P < 0.0001$). However, as one of the referees observed, there are concerns about the validity of this analysis because non-random covariation exists between the independent variable (heterozygosity) and 'population', hence, the relationship across population means.

A particular problem presented by UK rabbits is that their populations are genetically structured at extremely fine scales: sites in East Anglia separated by a few hundred metres are as different as those separated by 125 kilometres [4–6]. Such extreme differentiation means that insufficient genetic variation exists within discrete 'populations' to explore fitness relationships, with potential non-independence due to close relatedness.

Our results clearly indicate the established importance [7] of sampling very low heterozygosities for revealing possible fitness correlations: the relationship is heavily influenced by a small number (~12) of strongly homozygous males (one homozygous at all 29 loci). These individuals will be rare in the wild in general, and even rarer within one discrete population that holds individuals with a high degree of heterozygosity. Without an isolation-by-distance relationship, such fine-scale structuring also means that our sampling sites contain numerous 'populations'. As sampling was spread throughout most areas by several kilometres, we can be confident that most individuals represent single samples from different 'populations' by those criteria.

A key consideration is what constitutes a discrete population? There are arguments for designating the UK to be a population at one level of interpretation: rabbits colonised the wild recently, in the 18th century [4,8], providing us with a natural experiment. However, we agree that we cannot eliminate all potential confounds. Although our study was founded upon evidence that heavily inbred big cats have unusually elevated sperm abnormalities [9], we cannot exclude additional interpretations, such as that proposed by Slate and Pemberton [1]: abnormal sperm constrain reproductive output which leads to inbreeding. Ideally, we would match our natural environment correlations with experiments that applied tight 'environmental' control. However, complementary approaches are important because inbreeding depression could be influenced by both genetic and environmental variation [10].

References

1. Slate, J., and Pemberton, J. (2006). Does reduced heterozygosity depress sperm quality in wild rabbits (*Oryctolagus cuniculus*)? Curr. Biol. *16*, 790–791.
2. Gage, M.J.G., Surridge, A.K., Tomkins, J.L., Green, E., Wiskin, L., Bell, D.J., and Hewitt, G.M. (2006). Reduced heterozygosity depresses sperm quality in wild rabbits, *Oryctolagus cuniculus*. Curr. Biol. *16*, 612–617.
3. Webb, N.J., Ibrahim, K.M., Bell, D.J., and Hewitt, G.M. (1999). Natal dispersal and genetic structure in a population of the European wild rabbit (*Oryctolagus cuniculus*). Mol. Ecol. *4*, 239–247.
4. Surridge, A.K., Bell, D.J., and Hewitt, G.M. (1999). From population structure to individual behaviour: genetic analysis of social structure in the European wild rabbit (*Oryctolagus cuniculus*). Biol. J. Linn. Soc. *68*, 57–71.
5. Surridge, A.K., Ibrahim, K.M., Bell, D.J., Webb, N.J., Rico, C., and Hewitt, G.M. (1999). Fine-scale genetic structuring in a natural population of European wild rabbits (*Oryctolagus cuniculus*). Mol. Ecol. *8*, 299–307.
6. Surridge, A.K., Bell, D.J., Ibrahim, K.M., and Hewitt, G.M. (1999). Population structure and genetic variation of European wild rabbits (*Oryctolagus cuniculus*) in East Anglia. Heredity *82*, 479–487.
7. Balloux, F., Amos, W., and Coulson, T. (2004). Does heterozygosity estimate inbreeding in real populations? Mol. Ecol. *13*, 3021–3031.
8. Thompson, H.V., and King, C.M. (1994). The European Rabbit: History and Biology of a Successful Colonizer. (Oxford: Oxford University Press).
9. Barone, M.A., Roelke, M.E., Howard, J., Brown, J.L., Anderson, A.E., and Wildt, D.E. (1994). Reproductive characteristics of male Florida panthers: comparative studies from Florida, Texas, Colorado, Latin America, and North American zoos. J. Mammal. *75*, 150–162.
10. Keller, L.F., and Waller, D.M. (2002). Inbreeding effects in wild populations. Trends Ecol. Evol. *17*, 230–241.

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Variability in a taste-receptor gene determines whether we taste toxins in food

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TAS2R bitter receptors are thought to have evolved to detect toxins in plants and foods and to modulate ingestion of them [1]. Indeed, virtually every plant, edible or otherwise, contains toxins. Although this toxin-detector hypothesis of bitter taste is prevalent, there is no indication that TAS2R receptors detect specific toxins enmeshed within natural foods, a necessary link for the natural selection argument. Several expressed TAS2R receptors, however, are known to respond to pure solutions of toxins. For example, some variants of the antithyroid-toxin receptor hTAS2R38 respond to phenylthiocarbamide (PTC) and propylthiouracil (PROP) [2,3], compounds which contain a thiourea (N-C=S) moiety. We report here that genotypes of *hTAS2R38* specifically determine humans' bitterness perception of plants that synthesize glucosinolates, a class of anti-thyroid compounds that also contain the thiourea moiety.

The natural selection argument for detecting thyroid toxins in plants is supported by data in which sensitivity to PTC was shown to be associated with decreased risk of both goiter (thyroid enlargement) and central neural defects in an Andean community with endemic goiter; no such association was found in a neighboring community that was treated en masse with iodine injections [4]. Endemic goiter arises under conditions of low iodine ingestion as an adaptive response to maintain levels of thyroid hormones, which incorporate inorganic iodine in a process facilitated by thyroid peroxidase [5].