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## Letter to the Editor

### The clones need to return: A comment on Archer et al. (2003)

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Phenotypes are influenced by genes (nature), the environment (nurture), and the interaction between these (Alcock, 1998; McFarland, 1999; Goodenough et al., 2001). The relative influence of genes versus environment varies by trait along a continuum, ranging from undetectable to practically complete. To quantify each influence, several approaches are taken, one of which is to compare phenotypes of fraternal versus identical siblings (e.g. Bouchard Jr. et al., 1990). This experimental design provides mathematically calculable differences in genetic similarity among individuals so that the influence of environment is more easily quantified. (Although full sibs share the same amount of genetic information as do fraternal twins, the former have potentially been exposed to different foetal conditions in the womb, so that some environmental influences may already have been experienced; this makes fraternal twins a better control than full sibs.) Unfortunately, identical siblings may be comparatively rare for many animals, meaning sample sizes may limit use of this approach. Using a novel approach, Archer et al. (2003) used cloned pigs to assess the relative importance of genes on a series of porcine behaviours. Cloning offers exceptional potential to artificially increase sample sizes of unique genotypes. Results of several experiments led Archer et al. (2003) to conclude that there was similar variation in behaviour within families of cloned pigs and outbred pigs. However, we argue here that this conclusion is premature.

First and foremost, Archer et al. (2003) use a design that provides no true replication. Inference is using findings from a known situation to predict an unknown situation. Inferential statistics require statistically independent samples following well-established principles (e.g. Hurlbert, 1984; Rice, 1989). An essential principle is to use several independent replicates to generate statistical parameters. Archer et al. (2003) assessed the influence of genes on variation in pig behaviour using just four litters of pigs; two were cloned and two were from outbred pigs, and both cloned lineages came from the same sow. This design provides just one independent sample of a cloned lineage and two for the outbred lineages, a dataset simply not amenable to inferential statistics. Thus, statements in Archer et al. (2003), such as “the variability in behaviour among cloned animals”, are premature as they have only measured variability *within a single clone (litter)*. From this, they can only conclude that the variation within this particular clone differed from variation in the two outbred litters they chose. They violate statistical inference in using a single clone to suggest that clonal litters in general (as is implied by the wording given above) are

comparable in behavioural variation to outbred litters. An appropriate experimental design would use multiple sets or litters (e.g. 10) of cloned pigs, each from a genetically distinct sow, and compare the behavioural variation within each of these lineages to behavioural variation from 10 similar sets of outbred pigs. The issue of pseudoreplication has previously been addressed in this journal (e.g. Weary and Fraser, 1998), but this study provides a relatively clear example of the problem.

A second reason for scepticism relates to the variability in the data presented (e.g. Table 1 in Archer et al., 2003), suggesting considerable experimental noise. It is difficult to meaningfully compare variances without estimates of within-test, within-subject variability. With such high variability, one would expect the non-significant sign test they obtain (Section 3.4). Assuming the authors had sufficient sample sizes, they should have provided estimates of repeatability so that readers could better judge the robustness of the data. Part of the variation in the data could arise from including a runt in the behavioural assessments. Runts are physically and behaviourally different from their siblings (e.g. Ritacco et al., 1997; O'Connell and Beattie, 1999; Bauer et al., 2003), and usually at the bottom of any social hierarchy.

Once reasonable estimates of variability can be generated (using a properly replicated design, and sensitive and repeatable measures), the variation needs to be compared in the most appropriate way possible. Archer et al. (2003) use an *F*-ratio to compare the two variances, an approach “severely and adversely affected by sampling non-normal populations” (Zar, 1999, p. 139; see Van Valen, 1978, for alternative tests). The authors do not show the full distributions, but an examination of Figs. 1 and 2 in Archer et al. (2003) suggests that the distributions do deviate from normality.

The conclusions drawn by Archer et al. (2003) should be considered premature. Other experiments using the same approach may have promise, but it is also worth noting that generations of controlled breeding have likely depleted genetic variation in domestic pigs relative to their wild forbears; this makes domestic breeds less than ideal choices for research on questions pertaining to clonal versus outbred behavioural variation.

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Dave Shutler\*

Nic McLellan

*Department of Biology, Acadia University, Wolfville  
NS, Canada B4P 2R6*

Daniel M. Weary

*Animal Welfare Program, University of British Columbia  
Vancouver, BC, Canada V6T 1Z4*

\*Corresponding author. Tel.: +1 902 585 1354; fax: +1 902 585 1059  
*E-mail address:* dave.shutler@acadiu.ca

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