Inbred women in a small and isolated Swiss village have fewer children

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Introduction

Darwin married his cousin and feared that the poor health and early death of several of his ten children were the result of ‘a serious form of inheritance from my poor constitution’ (Burkhardt et al., 1997). He therefore ‘wished that the truth of the often repeated assertion that consanguineous marriages lead to deafness and dumbness, blindness, etc., should be ascertained’ (Darwin, 1887). Although his request for a large-scale investigation into the possible detrimental effects of consanguinity was turned down, since then, the effects of inbreeding have been subject of continuous investigation into a wide range of species, including humans (Cavalli-Sforza & Bodmer, 1971; Jain, 1976; Thornhill, 1993; Bittles et al., 2002; Keller & Waller, 2002).

Unlike Darwin, we now know that inbred offspring have an increased probability of receiving the same deleterious recessive allele from both of their parents and that they are more homozygous in general, both of which may result in reduced fitness, i.e. inbreeding depression (Charlesworth & Charlesworth, 1987). Although the evidence for inbreeding depression in plants and nonhuman animals keeps accumulating (Keller & Waller, 2002), the exact nature of its effect on fitness in humans, and on human fertility in particular, remains unclear and subject to debate (e.g. Bittles et al., 2002).

Adverse effects of inbreeding on a couple’s reproductive success have been found in the form of, for example, an increased rate of miscarriage or infant mortality and morbidity (Bittles & Neel, 1994; Ober et al., 1999; Bittles, 2001; Jorde, 2001). Interestingly, however, several of the studies that found a strong effect of inbreeding on fecundity found that consanguineous couples in fact have more, rather than fewer, children (Bittles et al., 1991; Hussain & Bittles, 1999; Blanco Villegas & Fuster, 2007). A number of nonbiological explanations for this, at first sight counter-intuitive, pattern have been put forward. For example, related couples may get married at an earlier age, have a lower level of education or may gain socio-economic advantages by keeping wealth and land within their (extended) family (Bittles et al., 1991; Al Husain & Al Bunyan, 1997; Hussain & Bittles, 1999; Blanco Villegas & Fuster, 2007). Furthermore, couples...
may have more children to compensate for a higher rate of infant mortality (referred to as reproductive compensation) (Hussain & Bittles, 1999; Ober et al., 1999; Overall et al., 2002). Alternatively, this positive association between inbreeding and fecundity has been argued to reflect the negative effects of outbreeding depression, in which the break-up of coadapted gene complexes or favourable epistatic relationships (Lynch, 1991) and an increase in maternal–foetal incompatibilities (Philippe, 1974) reduce fitness of more distantly related couples. In line with this, a number of recent studies have found a nonlinear relationship between fertility and a couple’s relatedness, with fertility being maximized at levels of inbreeding greater than zero, in both humans (Helgason et al., 2008), nonhuman animals and plants (Edmands, 2007).

Unlike most studies on nonhuman animals, which focus much of their attention on the effects of inbreeding at an individual level (and thus the degree of relatedness of their parents), most studies on the effects of inbreeding in humans solely test for an effect at the level of the couple. So, rather than testing for an association between a parent’s coefficient of inbreeding and fecundity, they compare the reproductive success of related and unrelated couples. Although this approach will detect effects of inbreeding on prenatal or neonatal viability, it misses the non-negligible effects of inbreeding that may only manifest themselves later in life, for example when inbred offspring become reproductively active themselves. Indeed, in a long-term study on the effects of inbreeding in adult Hutterites, an Anabaptist sect, Ober et al. (1999) found that although more and less inbred women had the same number of children, inbred women had longer interbirth intervals, as well as longer intervals to a recognized pregnancy. Similarly, Robert et al. (2009) found an effect on family size of the inbreeding level of the father, at least at later age, in a pre-industrial Canadian population.

Gaining a better understanding of the effects of inbreeding in humans requires estimates of inbreeding depression in relatively outbred and egalitarian populations while accounting for any confounding nonbiological factors as much as possible. Furthermore, rather than focusing solely on parental relatedness (consanguinity), the effects of inbreeding throughout the complete life cycle have to be taken into account. Here, we therefore use genealogical data from a small and isolated village in the Swiss Alps and simultaneously test for the effects of parental relatedness and both maternal and paternal inbreeding on family size.

**Materials and methods**

**Genealogical data**

Using data from, among others, parish and town registries, we were able to reconstruct genealogies for most of the families living in two adjacent small (between 500 and 700 inhabitants) and isolated villages in the southern part of the Swiss Alps (Cavergno and Bignasco). It is worth noting that, compared to bigger cities, such isolated mountain villages are relatively egalitarian and, until recently, have seen relatively little social and economic change.

For the great majority of individuals, we had information on the identity of their father and mother, as well as on their year of marriage, the number of children they had and how many of these children reached adulthood. The total number of children and the number of children who reached adulthood were highly correlated (Spearman rank correlation: \( r = 0.91, P < 0.0001 \)) and provided qualitatively and quantitatively very similar results. As the number of children who reached adulthood is less complete and accurate (for example owing to emigration), we present here only the results for the total number of children. Immigrant couples, which may have had children born outside the village, were not included in the analyses (also see below).

To anonymize the data, all individuals received a unique numerical identifier before any analyses were performed. We included all couples where the woman was born before 1954 (assuming a maximum age at the onset of menopause of 55), assuring our estimate of the number of children refers to completed family sizes. Those couples where the year of birth of the woman was not known were included if they had got married before 1974. A total of 28 couples in which either the husband or the wife had been married previously were excluded.

**Estimating relatedness and inbreeding**

The probability of two individuals sharing an allele that is identical by descent (IBD) for a given locus is given by the coefficient of kinship (IBK) and provides a measure of the degree of relatedness or consanguinity of any two individuals. The probability of an individual inheriting two alleles that are IBD is equal to the parental coefficient of kinship and is referred to as an individual’s coefficient of inbreeding (Falconer & Mackay, 1996, pp. 82–88). Using ENDOG v4.6 (Gutiérrez & Goyache, 2005), we calculated for all couples the coefficient of kinship with their partner (i.e. the coefficient of inbreeding of their offspring) as measure of their relatedness, here referred to as \( R \), as well as the coefficient of inbreeding of both parents (i.e. the coefficient of kinship of the parents’ parents), here referred to as \( F_{\text{maternal}} \) and \( F_{\text{paternal}} \).

Unlike their molecular counterparts, pedigree-based estimates of inbreeding and relatedness measure \( F \) and \( R \) relative to the individuals in the base population (i.e. all individuals with unknown parents), which are assumed to be unrelated and outbred. Consequently, the amount of information on an individual’s ancestors sets a limit to the minimum \( F \) that can be detected. Consequently, individuals with \( F = 0 \) for which limited pedigree
information is available may still be inbred, and couples with \( R = 0 \) may still be related.

Even to detect the closest form of inbreeding (offspring from a brother–sister or parent–offspring marriage, resulting in \( F = 0.25 \)), we need to be able to go back at least two generations. It is for this reason that studies typically only include those individuals for whom all four grandparents are known. However, in systems in which such close inbreeding is rare and most inbreeding events are relatively distant (as is the case in humans), a dataset including all individuals for which all four grandparents are known will still contain a very large number of noninformative zero inbreeding coefficients. While we may choose to further restrict the data set (e.g. including only individuals where all eight great-grandparents are known) to exclude more noninformative inbreeding coefficients equal to zero, this may also result in the exclusion of some highly informative close inbreeding events.

Here, we chose to include all parents and offspring with \( F > 0 \) in our analysis, irrespective of the amount of pedigree information available. Additionally, we used ENDOG to calculate for all parents and their offspring the number of equivalent generations on which their inbreeding coefficient was based, providing a standardized measure of the amount of pedigree information available (Maignel et al., 1996; also see González-Recio et al., 2007). Following González-Recio et al. (2007), we additionally included all parents and offspring with \( F = 0 \) for which all four grandparents were known and for which the number of equivalent generations was 4 or more. Note that the results were relatively insensitive to the exact location of this cut-off value and that any cut-off value between three and five equivalent generations provided similar results. Furthermore, including only individuals with \( F > 0 \) and/or couples with \( R > 0 \) (so excluding all \( F = 0 \) and \( R = 0 \) or excluding all estimates of \( F \) and \( R \) based on less than that four equivalent generations (irrespective of whether \( F \) or \( R \) are greater than zero) resulted in smaller datasets but provided quantitatively very similar results.

Given that, as pointed out earlier, inbreeding coefficients are dependent on pedigree depth. González-Recio et al. (2007) suggested to regress fitness against the increase in inbreeding per generation (\( \Delta F \)), rather than against \( F \) itself. However, in our case, \( F \) and \( \Delta F \) were strongly correlated (\( \tau > 0.9 \)), and the use of \( \Delta F \) instead of \( F \) provided virtually identical results. As the interpretation of the slope of the regression of fitness against \( \Delta F \) (or \( \Delta R \)) is less straightforward, we present the results for \( F \) (and \( \Delta R \)) only.

**Statistical analyses**

We tested for an effect of the relatedness of a couple (\( R \), i.e. the level of inbreeding of their children), as well as both the paternal and the maternal level of inbreeding (\( F_{\text{paternal}} \) and \( F_{\text{maternal}} \), respectively) on the total number of children they had (\( N_{\text{total}} \)). \( N_{\text{total}} \) was square-root transformed \( \sqrt{(N_{\text{total}} + 1)} \), as suggested by a Box–Cox transformation. Shapiro–Wilk tests for normality showed no evidence for significant deviations from normality of the residuals for any of the models.

Analyses using the full data set showed a significant decline in family size over time (year of marriage: \( b \pm SE = -0.0030 \pm 0.00058, \ P < 0.001 \)). Year of marriage was therefore included as a covariate in all models. Furthermore, to account for the possible nonindependence of brothers and sisters (who have the same inbreeding coefficient and share a household for the early parts of their lives), we included paternal and maternal family (defined as a unique combination of the ID of both parents of the husband and the wife, respectively) as random effects in all models (irrespective of their statistical significance). Whereas in the full data set (so including couples with insufficient pedigree information, see above) paternal family explained a significant 31% of variation in \( N_{\text{total}} \) (\( \chi^2 = 20.9, \ P < 0.001 \)), maternal family explained as little as 6.0% (\( \chi^2 = 6.01, \ P = 0.438 \)). However, although paternal family explained quantitatively similar amounts of variation in the more restrictive models including \( R \) or \( F \) (see below), it never reached statistical significance in these models.

We first tested simultaneously for an effect of \( R \), \( F_{\text{paternal}} \) and \( F_{\text{maternal}} \) on \( N_{\text{total}} \). Note that sample sizes are greatly reduced for this model as only couples for which we have sufficient pedigree information for both the husband and the wife (and thus for their offspring) could be included (see above). Additionally, we therefore fitted models including \( R \), \( F_{\text{husband}} \) and \( F_{\text{wife}} \) separately.

All statistical analyses were performed using R version 2.9.1 (R Development Core Team, 2008). Linear mixed models were run using the lme4 package (Bates et al., 2008). The significance of the fixed effects as well as their 95% confidence intervals was obtained from 100.000 MCMC simulations, performed using the pvalsInc function in the languageR package (Baayen, 2007). Significance of the random effects was assessed using likelihood ratio tests.

**Results**

**Descriptive statistics**

In total, we had data on completed family size for 465 couples. The great majority of these couples got married in the 19th century (earliest: 1745; 1st quartile: 1820; median: 1851; 3rd quartile: 1894; latest: 1950). The median completed family size was 4 (minimum: 0; 1st quartile: 2; 3rd quartile 7; maximum: 16).

The distribution of parental and offspring \( F \), as well as the amount of pedigree information on which these estimates are based, is depicted in Fig. 1. It is clear from
When we included Effects of parental relatedness and inbreeding (0.0194 across couples with sufficient pedigree information available, given by the coefficient of kinship of their parents). The amount of pedigree information on which an individual’s inbreeding coefficient was based is expressed in the equivalent number of generations. In grey are the coefficients of inbreeding that were excluded because of insufficient pedigree information. For more information, see Materials and methods.

this figure that, provided that there is sufficient pedigree information available, all individuals are to some degree inbred (i.e. \( F > 0 \)), albeit often only very little. The data points that were excluded from subsequent analyses (\( F = 0 \) and equivalent number of generations < 4, also see Materials and methods) are depicted in grey.

The mean inbreeding level across all men was 0.0063 and across those that fitted our criteria for inclusion mean \( F \) was 0.0197 (\( n = 74 \)). Mean \( F \) across all women was lower (0.0030) but similar when only women with sufficient pedigree information were included (0.0190, \( n = 148 \)). The latter is because there are relatively more women than men for which we cannot obtain an informative estimate of \( F \) because their parents and grandparents are unknown. This may be the result of female-biased immigration or of the fact that a woman’s birth name was not always recorded when she got married. Mean \( R \) was 0.0065 across all couples and 0.0194 across couples with sufficient pedigree information (\( n = 153 \)).

**Effects of parental relatedness and inbreeding**

When we included \( R \), \( F_{\text{paternal}} \) and \( F_{\text{maternal}} \) in the model, we found no effect of either parental relatedness or paternal inbreeding on family size. However, there was a significantly negative effect of the inbreeding level of the mother on the total number of children she had throughout her life (Table 1, Fig. 2). Furthermore, family sizes were found to have declined significantly over time, and paternal but not maternal family explained a substantial (but nonsignificant) proportion of the variation in family size (Table 1). There was no significant quadratic effect of year of marriage \((P = 0.33)\), and inclusion of the quadratic term provided very similar estimates for the effects of relatedness and inbreeding.

To maximize statistical power, we subsequently tested for an effect of \( R \), \( F_{\text{paternal}} \) and \( F_{\text{maternal}} \) in three separate models. Again, we found a significantly negative effect of maternal inbreeding level on family size \((b \pm \text{SE} = -9.02 \pm 3.02, \ P = 0.0036)\), but no effect of parental relatedness \((b \pm \text{SE} = 1.11 \pm 2.29, \ P = 0.55)\) or paternal inbreeding level \((b \pm \text{SE} = -1.66 \pm 2.40, \ P = 0.41)\). Furthermore, there was no evidence for a quadratic effect of either parental relatedness \((P = 0.83)\), maternal inbreeding \((P = 0.70)\) or paternal inbreeding \((P = 0.78)\), although it should be noted that statistical power was very low. In all three models, there was again a highly significant effect of the year of marriage, and again, paternal but not maternal family explained a substantial, but nonsignificant, amount of variation in \( N_{\text{total}} \) (results not presented).

**Discussion**

Most studies on the effects of inbreeding in humans have treated the couple as the unit of interest and hence tested solely for an association between the degree of relatedness of a couple \((R)\) and their reproductive success. This is in contrast to studies on nonhuman animals, which typically focus much more on the effects of inbreeding \((F)\) on an individual level. Here, we combined these two approaches and tested whether couples in which husband and wife are closely related have fewer children than more distantly related couples and, at the same
time, whether there is an effect of the inbreeding levels of the two parents themselves.

Our finding that inbred mothers have fewer children, but inbred fathers do not, is in accordance with the fact that all prenatal and a large proportion of the postnatal parental investment is provided by the mother. Consequently, the number of genes that are involved in parental investment and/or care that is expressed in women is many times higher than in men, all of which may carry deleterious recessive alleles that may be expressed in inbred individuals. This finding is also in line with studies that have quantified the effects of paternal and maternal inbreeding in nonhuman animals. In great tits (Parus major), for example, hatching success depends not only on the inbreeding level of the egg, but also on that of the mother (Van Noordwijk & Scharloo, 1981). Similarly, in song sparrows (Melospiza melodia), inbred females, but not inbred males, have a lower lifetime reproductive success (Keller, 1998). Although inbreeding may well have negative effects on sperm quality and quantity, and thereby reduce fecundity of inbred males (Roldan et al., 1998; Asa et al., 2007; Fitzpatrick & Evans, 2009), this may only become important in species where the level of sperm competition is high (Simmons et al., 2004; Zajitschek et al., 2009).

Furthermore, there is some evidence that the negative effects of inbreeding on sperm quality or quantity in humans may only become apparent at a relatively late age (Robert et al., 2009). Although the differential effect of maternal and paternal inbreeding makes biological sense, it should be noted that statistical power is relatively weak (as illustrated by the 95% confidence intervals in Table 1), and hence, we cannot exclude the existence of a negative effect of paternal inbreeding. Furthermore, the absence of an effect of paternal inbreeding, and of parental relatedness for that matter, could also be explained by extra-pair paternity (see Simmons et al., 2004), which introduces errors in the pedigree along the paternal line. Nevertheless, although this may account for the absence of an effect of paternal inbreeding and parental relatedness, it cannot explain the significantly negative effect of maternal inbreeding on reproductive success. In fact, pedigree errors along the paternal line would indirectly also affect the accuracy of the maternal inbreeding coefficients, making our estimate of the strength of maternal inbreeding depression, if anything, more conservative.

Our main interest here lies in the evolutionary consequences of inbreeding in humans, i.e. in the association between parental relatedness and inbreeding, and fitness. Although we find that inbred women have fewer children, the data do not allow us to further dissect the causality of this relationship. Hence, we can only speculate about why maternal inbreeding reduces family size. Inbred women may, for example, experience a higher risk of miscarriage or have lower conception rates or higher peri-implantation loss rates (Ober et al., 1999). More indirectly, inbreeding may reduce female reproductive success through, for example, the age at marriage. If women who marry at an earlier age have more children, we may find a negative effect of inbreeding on family size if inbred women typically marry at a later age. Indeed, we find a significantly negative effect of the maternal age at marriage on completed family size (b = −0.041 ± 0.0079, P < 0.0001), but not of paternal age at marriage (b = −0.0086 ± 0.0065, P = 0.51). Yet, there is only a weak and nonsignificant positive effect of inbreeding on maternal age at marriage (b = −0.061 ± 0.039, P = 0.20) (note that age at marriage was reciprocally transformed to improve normality). Furthermore, when we include maternal age at marriage as an additional covariate in the model testing for an effect of maternal inbreeding on family size, the effect of maternal inbreeding is reduced only slightly (maternal inbreeding: b = −7.24 ± 3.10, P = 0.022; maternal age at marriage: b = −19.08 ± 14.8, P = 0.046). Although this suggests that age at marriage might at least partly contribute to the negative effect of inbreeding on
reproductive success in women, again we can only speculate about the underlying mechanism.

Unlike earlier studies on the effects of inbreeding in humans, we did not find that related couples had fewer (or more) children (Bittles & Neel, 1994; Bittles, 2001; Jorde, 2001). However, the great majority of studies on the effects of inbreeding in humans have been performed in communities with exceptionally high rates of inbreeding in, among others, India (mean $F = 0.01–0.05$) and Pakistan (mean $F = 0.02–0.03$) (Bittles et al., 2002). Importantly, these countries are also characterized by large amounts of, potentially confounding, variation in socio-economic status (Hussain & Bittles, 1999, 2002; Bittles et al., 2002). Estimates of the effect of inbreeding in less inbred and more homogeneous societies remain however scarce (but see Ober et al., 1999; Helgason et al., 2008; Robert et al., 2009). The few studies using data from populations more similar to the one described here (i.e. more homogenous and egalitarian, and less inbred) typically found small and often complex effects of a couple’s relatedness on total number of children or no effect at all (Ober et al., 1999; Overall et al., 2002; Helgason et al., 2008; Robert et al., 2009). The absence of a strong negative effect of inbreeding in this study may at least partly be attributed to reproductive compensation, especially if family sizes are smaller than the reproductive maximum (Ober et al., 1999; Overall et al., 2002). However, our finding that more inbred mothers do indeed have fewer children, and thus do not show complete compensation, suggests that reproductive compensation may not be the sole explanation. Furthermore, several recent studies on humans and other animals have found significantly nonlinear effects of relatedness on reproductive success, with reproductive success being maximized at intermediate levels of relatedness (Edmands, 2007; Helgason et al., 2008). Although here we do not find any significantly nonlinear effects, statistical power is very low.

Although it will never be possible to completely rule out the role of nonbiological factors in driving the observed negative effect of maternal inbreeding found here, it should first of all be noted that, except for a few cases (two men, who were brothers, and one woman with $F = 0.125$), we find very little close inbreeding, and many couples with $R > 0$ would probably have been unaware of the fact that they were related. Given that most inbreeding events are relatively distant, correlations between their relatedness and their socio-economic status are expected to be very weak at most. Second, we tested for an effect of parental relatedness and both maternal and paternal inbreeding simultaneously. As we can expect socio-economic and other nonbiological effects (e.g. giving rise to systematic year-to-year differences in inbreeding level and family size) to act on the family level (i.e. on both the wife and the husband), we would expect to find similar effects of maternal and paternal inbreeding, and of parental relatedness. Third and finally, by including maternal and paternal family as random effects in our model, we were able to control at least partly for the effects of variation in cultural or socio-economic differences among families. With respect to the latter, it is interesting to note that whereas we found paternal family to explain a substantial and significant proportion of the variation in family size, there was no effect of the maternal family on a couple’s reproductive success. In other words, brothers had similar family sizes, whereas sisters did not. One potential explanation for this pattern could be the inheritance system in which sons benefit more from the wealth of their parents than daughters.

Although social scientists continue to prefer nonbiological explanations (e.g. Shor & Simchay, 2009), evidence in support for the idea that the taboo on incest that exists in many societies has evolved as a mechanism to avoid inbreeding depression continues to accumulate (Maynard Smith, 1978; Lieberman et al., 2003). However, whereas the latter assumes that mating with a relative results in fewer offspring, we found that closely related partners did not have smaller families than those that were more distantly related. Importantly, however, we found that the daughters of parents who are closely related (that is daughters who were inbred themselves) did have fewer children than those with less closely related parents. Consequently, although related parents do not have fewer children, their inbred daughters do, providing further evidence for selection for inbreeding avoidance, also in humans.

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References


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