ORIGINAL ARTICLE

Outbreeding and possibly inbreeding depression in a pollinating fig wasp with a mixed mating system

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Mixed mating systems are somewhat of an enigma as most models predict that organisms should either inbreed when inbreeding depression is low, or outbreed when inbreeding depression is high. Many wasps mix routine inbreeding with a little random mating. This random mating is most common when all local sibmating opportunities are exhausted and dispersal is the only way males can further increase their fitness. The males of the pollinating fig wasp, *Platyscapa awekei*, are slightly different in that they disperse before all sibmating opportunities have been exhausted. To see if this is a response to inbreeding depression we quantify inbreeding depression by comparing females' life time reproductive

success to their heterozygosity at multiple microsatellite loci. We find that a female wasp's heterozygosity is an accurate predictor of her inbreeding coefficient and that *P. awekei* females actually seem to suffer from outbreeding depression and possibly from a little inbreeding depression. Male dispersal is thus not a means to effect the optimal mating system, but more likely a mechanism to reduce competition among brothers. The number of mature offspring a female produces depends on her own heterozygosity and not on that of the offspring, and may be determined by egg and gall quality. *Heredity* (2009) **102**, 349–356; doi:10.1038/hdy.2009.2; published online 18 February 2009

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Introduction

In a large number of organisms individuals either avoid or prefer relatives as mating partners (Hamilton, 1967; Maynard Smith, 1978; Greenwood, 1980; Schemske and Lande, 1985; Charlesworth and Charlesworth, 1987). This dichotomy in mating systems, either in- or outbred, is in line with most models that show that below a threshold value of inbreeding depression organisms should inbreed, and above the threshold, they should avoid inbreeding (Bengtsson, 1978; Parker, 1979; Holsinger *et al.*, 1984; Lande and Schemske, 1985; Charlesworth *et al.*, 1990; Taylor and Getz, 1994; Kokko and Ots, 2006). Inbreeding is favoured because of its transmission advantage (Fisher, 1941), which can be seen as a kin-selective advantage (Bengtsson, 1978; Cowan, 1979; Parker, 1979).

Even so, a not insignificant number of organisms mix these two strategies (Jain, 1976; Hardy, 1994; Godfray and Cook, 1997; Goodwillie *et al.*, 2005). These mixed mating systems are a mixture of random mating and either selfing (in the case of hermaphrodites) or sibmating. Theory suggests that mixed mating can be stable under a variety of circumstances (Maynard Smith, 1977; Lloyd, 1979; Campbell, 1986; Uyenoyama, 1986; Charlesworth

and Charlesworth, 1990; Uyenoyama and Waller, 1991a,b; Uyenoyama *et al.*, 1993; Taylor and Getz, 1994).

Outbreeding depression is the reduction in fitness when partners are too distantly related and may influence the evolution of mating systems and dispersal (Price and Waser, 1979). A number of empirical studies on a diverse array of animals have found outbreeding depression within animal populations (*Caenorhabditis elegans*: Dolgin *et al.*, 2007; bark beetles: Peer and Taborsky, 2005; ornate dragon lizard: LeBas, 2002; song sparrow: Marr *et al.*, 2002), sometimes in conjunction with inbreeding depression (humans: Helgason *et al.*, 2008; Arabian oryx: Marshall and Spalton, 2000; fish: Neff, 2004; *Daphnia*: de Meester, 1993). When both inbreeding and outbreeding depression are taken into account, it seems logical that there should be a preference for mating partners that are moderately different from oneself, which would constitute the optimal outbreeding distance (Price and Waser, 1979; Bateson, 1983).

A lack of gene flow is required to build up the genetic incompatibilities that cause outbreeding depression. Schierup and Christiansen (1996) showed that limited dispersal can result in an optimal outbreeding distance. Ironically, the limited range of dispersal required to build up sufficient levels of co-adaptation to result in outbreeding depression, is smaller than the optimal outbreeding distance, as was foreseen by Waddington (1983). This type of 'frustrated' mating system may be very common; in parasitoid wasps that frequently have mixed systems (Hardy, 1994), the mating options are the extremes of sibmating or outbreeding, not some intermediate level. Similarly, humans, for whom the optimal outbreeding distance seems to be third and fourth

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cousins (Helgason *et al.*, 2008), have cultures where unrelated or first cousin marriages are the norm (Bittles, 2001). Mixed mating systems may be a 'frustrated' means to balance the evils of inbreeding and outbreeding depression.

Mixed mating systems are frequent in parasitoid Hymenoptera (Hardy, 1994; Godfray and Cook, 1997) and haplodiploids in general (Werren, 1993; Normark, 2004). In haplodiploid species recessive deleterious alleles cannot hide in haploid males and are continuously purged from the population (Brückner, 1978). Therefore theory suggests (Werren, 1993) and data concur (Antolin, 1999; Henter, 2003) that the degree of inbreeding depression is less in haplodiploids than in diploids. In fact, researchers frequently fail to show any inbreeding depression (Biémont and Bouletreau, 1980; Clarke *et al.*, 1992; Sorati *et al.*, 1996; Peer and Taborsky, 2005). Even so, inbreeding depression can be substantial in haplodiploids (Henter, 2003), presumably because of a reduction in the benefits of overdominance (Lynch and Walsh, 1998). The most common reason for inbreeding depression in Hymenoptera is complementary sex determination (Cook, 1993), which is effectively a form of overdominance.

Pollinating fig wasps are renowned for their highly inbred lifestyle but many species frequently have a mixed mating system (Herre *et al.*, 1997; Greeff, 2002; Molbo *et al.*, 2002, 2004; Zavodna *et al.*, 2002; Greeff *et al.*, 2003; Jansen van Vuuren *et al.*, 2006). The mixed mating system of fig-pollinating and many parasitoid wasps (Hardy, 1994; Godfray and Cook, 1997) may be sub-optimal, but still the best response from a set of inferior options. For instance, where more than one mother oviposits in a patch (or fig), an inability to discern kin from non-kin will inevitably result in outbreeding. Further, when most females in a patch are mated, or when there are only male offspring on a patch, males' only option is to disperse and mate with unrelated females. Consequently, even though sibmating may be the best strategy, outbreeding may frequently be males' only option to increase their own fitness.

In this context, the pollinating fig wasp, *Platyscapa awekei*, is interesting because its males start to leave figs well before all the females are mated (Moore *et al.*, 2006). Moore *et al.* (2006) concluded that such dispersal may help to reduce mating competition between brothers. An alternative explanation is that there may be an advantage to outbreeding, perhaps the avoidance of inbreeding depression. The actively pursued mixed mating system of *P. awekei* indicates that it will be informative to measure if it suffers from in- or outbreeding depression.

Many studies have used the correlation between heterozygosity at multiple loci and fitness of individuals to assess inbreeding depression (Hansson and Westerberg, 2002). These studies have been criticized because heterozygosity is often a very poor estimate of the inbreeding coefficient (Hansson and Westerberg, 2002; Balloux *et al.*, 2004; Slate *et al.*, 2004). However, all these authors agree that if there is a mixed mating system that results in a high variance in the inbreeding coefficient, multi-locus heterozygosity may in fact be a good reflection of the level of inbreeding (Pemberton, 2004).

In this study, working on *P. awekei*, we (1) show that multi-locus heterozygosity is a good surrogate for the inbreeding coefficient, (2) quantify the effect of a

mother's inbredness on her life time production of mature offspring and (3) quantify the effect of a female's inbredness on her own size and egg complement.

Materials and methods

Relevant fig wasp biology and the study species

The relevant details of a fig wasp's life history can be summarized as follows (Hamilton, 1979; Herre *et al.*, 1997; Kjellberg *et al.*, 2005): mated, pro-ovigenic females disperse to trees containing receptive figs. The fig is an inflorescence composed of many uni-ovulate flowers facing towards the inside cavity of the fig. A single or a few females crawl into the figs and are trapped inside and are thus obliged to lay all her eggs in a single fig (but see Moore *et al.*, 2003 for exceptions). Inside the females oviposit all their eggs, one egg per flower and the mothers gall each flower to support the developing larvae. Larvae hatch and feed on the gall and eventually pupate within it. Males eclose from their galls first and search for galls containing females into which they chew a mating hole through which they mate with the females. The successful development of a wasp will thus depend on its own genes but also on the quality of the egg it hatched from and on the gall in which it developed. The egg and gall quality are most probably determined by the mother. In most species mating takes place inside the fig of origin. If mating takes place at random in the fig, there will be out- and inbreeding as soon as more than one female contributed to the offspring in the fig. In the species studied here the average foundress number is 1.9 females per fig with 55% of all figs containing single foundresses (DVK Newman and JM Greeff, unpublished data). Zavodna *et al.* (2005) suggested that *Liporrhopalum tentacularis* mothers' oviposition patterns will in fact encourage outbreeding in multiple foundress figs, whereas Frank (1985) gave indirect evidence for increased sibmating in *Pegoscapus assuetus*. The males of *P. awekei* and a number of other species disperse actively to neighbouring figs and this will result in outbreeding (Greeff *et al.*, 2003). Not one of 60 single foundress figs contained only male offspring suggesting that the frequency of only male figs are very low (DVK Newman and JM Greeff, unpublished data).

Sibmating and homozygosity

Loci can be homozygous through identity by state (ibs) and identity by descent (ibd) and the magnitude of each will depend on the inbreeding coefficient (F) and the homozygosity expected under panmixia:

$$P(\text{homozygous}) = P(\text{ibd}) + P(\text{ibs}|\text{not ibd}) \cdot P(\text{not ibd}) \\ = F + P(\text{ibs}|\text{not ibd}) \cdot (1 - F) \quad (1)$$

When there is little variation in F , most of the variance in $P(\text{homozygous})$ is caused by variation in $P(\text{ibs}|\text{not ibd})$ and then heterozygosity is a poor predictor of F (Pemberton, 2004). As we want to use homozygosity as a proxy for F , it is important to first get an appreciation for how closely they are linked in *P. awekei*. Slate *et al.* (2004) give equations (their equations (2) and (4)) to calculate the correlation between heterozygosity and F . The parameters these equations require are the expectation of F , the $\sigma^2(F)$, the number of loci studied and the heterozygosity at these loci. From Jansen van

Vuuren *et al.* (2006) we know that the expectation of F for *P. awekei* is equal to 0.423. Next we need to calculate the variance in F .

Given a life history where females either sibmate or breed out, a sibmating rate of about 0.778 will result in a population-wide F of 0.423 (Suzuki and Iwasa, 1980). Assuming this rate is independent of a female's F , it is easy to calculate the frequency of classes of individuals that are outbred (P_0), once sibmated (P_1), two times sibmated (P_2) and so on. Then $\sigma^2(F) = \sum P_i (F_i - \bar{F})^2$, with F_i equal to F of a female whose immediate ancestors have sibmated uninterruptedly for i generations. With successive generations of inbreeding, the increments in F for haplodiploids are the same as for diploids (Stubblefield and Seger, 1990) and are given by:

$$F_i = \frac{F_{i-1}}{2} + \frac{F_{i-2}}{4} + \frac{1}{4} \quad (2)$$

with $F_{-1} = F_0 = 0$ (Lynch and Walsh, 1998). Note that in haplodiploids the inbreeding coefficient refers to that expected in the diploid females and not in the males who are haploid.

The expected heterozygosity under panmixia for the six loci studied here: 4, 1, 21, 32, 7 and 8 (Jansen van Vuuren *et al.*, 2006) were 0.912, 0.833, 0.833, 0.831, 0.833 and 0.833, respectively. Owing to the uniformity of the expected heterozygosity over loci, we used the average of 0.846.

If we parameterize the equations of Slate *et al.* (2004) with these estimates we find that the correlation between the proportion of loci we find heterozygous and F of an individual is -0.83 .

To appreciate the noise involved in this estimate it is instructive to plot it. We set the homozygosity to 0.154 and used a binomial distribution to approximate the distribution of observing nought to six loci out of six to be homozygous under panmixia. Combining this binomial distribution and equation (2) with equation (1) gives the expected frequency distribution of the number of loci homozygous for this population of fig wasps over successive generations of sibmating (Figure 1; this was confirmed with Monte Carlo simulations). As F increases over successive generations of inbreeding the expected number of loci that will be homozygous also increases (Figure 1). Even though the expectation of the number of

loci homozygous as a function of the inbreeding coefficient is a straight line (Figure 1b), variation (Figure 1a) reduces the correlation to 0.83.

Number of offspring reaching adulthood

The number of offspring that reaches adulthood is a common measure of fecundity (Henter, 2003) even though it can be confounded by the genetic composition of the offspring. All experiments took place in the National Botanical Gardens in Pretoria and the gardens of the University of Pretoria. The experiment was replicated three times on different trees and at different times: Tree 1, April 2002; Tree 2, November 2002 and Tree 3, January 2003. It would thus not be possible to distinguish the effects of time and fig tree, but their combined effect can be estimated. Figs to be used in the introduction experiments were bagged with mesh material bags two to three weeks before they became receptive. This prevented non-experimental females from entering the figs. Once figs on the recipient tree became receptive, we collected experimental wasps as follows: figs from donor trees were picked and placed in 200 ml plastic bottles. On average 50–100 such figs per tree were collected from three to five trees. Female wasps were collected from the bottles with a brush and placed upon a receptive fig. After the wasp had entered the fig we closed the osteole with Pratley putty to prevent non-experimental wasps from entering. In trees 1, 2 and 3, we introduced 20, 10 and 70 wasps, respectively. After the introductions the figs were bagged again. Two weeks later the putty was removed from the osteole to ensure unhampered fig development. Just before releasing their wasps, the figs were picked and placed individually into plastic containers. Released wasps were transferred to 1.5 ml Eppendorf tubes containing 95% EtOH. The figs were carefully dissected under a dissection microscope and any additional wasps found were added to the released wasps. The wasps were collected while still alive, as initial microsatellite analysis showed that dead wasps did not yield good quality template DNA. Clutch size and sex ratio were counted from the wasps from each fig. A sample of the offspring were genotyped to reconstruct the genotype of their mother (see Genotyping).

Figure 1 The relation between the successive generations of inbreeding and the number of loci homozygous. (a) Frequency at which a certain number of alleles (0–6) can be expected to be homozygous. Starting at the top from an outbred individual with nine successive generations of sibmating as we move down the panels. (b) The relationship between number of loci homozygous and the inbreeding coefficient. The successive points starting from bottom left show the increase in the mean inbreeding coefficient over successive generations of sibmating.

Wasp size and egg number

Female wasps for this experiment were collected from the National Botanical Gardens in Pretoria in February 2003. Females from different figs were used to ensure independence of samples. Ninety-three wasps were dissected under the microscope and their ovaries taken out and placed on a glass slide. The eggs of the two ovaries were counted separately (see Kathuria *et al.*, 1999 for details). Tibia length was measured under the microscope. The remainder of the wasps were used for DNA extraction and genotyped (see Genotyping).

Genotyping

The haploid males get all their alleles from their mothers. Therefore, by genotyping sons it is possible to reconstruct the mother's genotype. When a set of brothers carries either of the two alleles at a locus the mother must have been heterozygous and when they carry one allele the female may well have been homozygous, but there is a slight chance that she was heterozygous. If N males are genotyped and all carry the same allele, then the chance that a mother is in fact heterozygous is $(1/2)^N$. Therefore when at least six males have been genotyped, the chance that a mother that is heterozygous at the specific locus is incorrectly classed as homozygous, is less than 1.6%. In few cases where too few males were available, we genotyped additional daughters to see if the allele was ever not present. In this way loci were classed as homozygous or heterozygous and were tallied for each female.

All samples were genotyped on ABI 3100 automated sequencer and genemapper software. DNA extractions were done using the Chelex extraction protocol (Estoup *et al.*, 1996). Six microsatellite loci developed for *P. awekei* (Jansen van Vuuren *et al.*, 2006) were amplified in four reaction mixtures: loci 1, 32 and 4 on their own and loci 7, 8 and 21 together. Here, 10 μ l reaction volumes composed of 1 $\times$ PCR buffer with $MgCl_2$, 800 nM dNTP's, 0.7 U Taq Polymerase and between 583 and 1000 nM of primer were set up. The PCR conditions consisted of initial denaturation, 2 min at 95 °C with 30 cycles of 40 s at 95 °C, 1 min at 60 °C and 2 min at 72 °C. No final elongation step was used so as to avoid stutter peaks. Reactions were cooled to 4 °C and stored at this temperature until diluted for genescan. All amplifications were diluted 20:1 with UHQ; different primer amplifications were mixed in a ratio of 2:1:1:2. Amplifications were genotyped and the results analysed with genemapper software.

Statistics

All statistics were done in R ver 2.6.1 (R Development Core Team, 2007). Clutch size was modelled with a linear model of tree and its interaction with the number of alleles homozygous and its square (Clutch size $\sim$ Tree ($\eta + \eta^2$), where η refers to the number of homozygous loci). This model was reduced by deleting non-significant terms, starting with the interactions and working down to find the minimum adequate model. A visual inspection of the data suggested that we should also fit clutch size as a function of tree and its interaction with the log of the number of homozygous loci (Clutch size $\sim$ Tree $\times$ Ln($\eta + 1$)). Again a linear model was fitted and the minimum adequate model was found in the same way. For the second experiment wasp size was first modelled as a dependent variable of η and η^2 . Then egg

number was modelled as a function of homozygosity and wasp size. Finally, as fluctuating asymmetry may reflect imbalances in gene complexes due to in- or outbreeding (Møller and Swaddle, 1997), the absolute value of difference in egg number between left and right ovaries, divided by the average number of eggs for the two ovaries, was modelled as a function of η and η^2 . Again linear models were used and non-significant terms were deleted until the minimum adequate model was obtained.

Results

The variance in the number of homozygous loci was similar in the two experiments, 3.619 and 3.271, respectively.

Number of offspring reaching adulthood

From trees one to three we successfully harvested and genotyped the offspring of 17, 10 and 30 mothers, respectively. One fig that had a clutch size of just over two times that of the mean, suggesting that two females entered the fig, was very influential on the statistical models and was deleted from the analyses. When this fig was included, one additional model to the two discussed below, namely: Clutch size $\sim$ Tree $\times \eta$, was also significant. The deletion or inclusion of this outlier fig did not affect the other two models' significance or parameter estimates much. Two models explained the data almost equally well (Table 1; Figure 2). In model 1 the clutch sizes for trees one to three (c_1 , c_2 and c_3) were best explained as a function of the tree, the number of homozygous loci (η) and η^2 (Table 1):

$$\begin{bmatrix} c_1 \\ c_2 \\ c_3 \end{bmatrix} = \begin{bmatrix} 39.98 \\ 38.63 \\ 26.07 \end{bmatrix} + 9.71 \times \eta - 1.31 \times \eta^2$$

In the second model clutch size was best explained as a factor of the tree and Ln($\eta + 1$):

$$\begin{bmatrix} c_1 \\ c_2 \\ c_3 \end{bmatrix} = \begin{bmatrix} 42.42 \\ 41.24 \\ 29.55 \end{bmatrix} + 7.78 \times \text{Ln}(\eta + 1)$$

Both these models suggest that there is in fact outbreeding depression, whereas model 1 suggests some

Table 1 ANOVA table for models 1 and 2

Source	d.f.	F	P
<i>Model 1: adjusted R² = 0.215</i>			
MAM terms			
Tree	2	5.766	0.005
Homozygous	1	6.989	0.011
Homozygous ²	2	4.845	0.032
Deleted terms			
Tree:homozygous	2	1.432	0.249
Tree:homozygous ²	2	2.073	0.137
<i>Model 2: adjusted R² = 0.189</i>			
MAM terms			
Tree	2	4.915	0.011
Ln(homozygous+1)	1	5.518	0.023
Deleted terms			
Tree:Ln(homozygous+1)	2	1.659	0.201

Homozygous = the number of loci that were scored as homozygous; MAM = minimum adequate model.

Figure 2 The relationship between clutch size and number of homozygous loci. Tree 1, circles and dashed lines; tree 2, triangles and dotted lines; tree 3, squares and solid lines; Model 1, heavy lines; Model 2, thinner lines.

additional inbreeding depression. The trees differ markedly in the number of offspring produced.

Wasp size and egg number

The 87 wasps that were successfully counted and typed carried 53.07 (s.d. = 8.66) eggs on average. The level of inbreeding had no effect on the size of the wasp (homozygous: $F_{1,86} = 0.355$ $P = 0.553$; homozygous²: $F_{1,86} = 0.284$, $P = 0.595$). The number of homozygous loci also had no effect on the wasp's egg load ($F_{1,85} = 0.401$, $P = 0.528$), whereas the wasp's size strongly co-varies with her number of eggs ($F_{1,86} = 192.6$, $P < 0.001$, adjusted $R^2 = 0.69$). The inbredness of female wasps did not explain the asymmetry in the number of eggs in their two ovaries (homozygous loci²: $F_{1,85} = 0.690$, $P = 0.408$; homozygous loci: $F_{1,86} = 2.480$, $P = 0.119$).

Discussion

In general we found that the life time number of mature offspring under natural conditions depends on the inbredness of the mother, whereas the size, egg load and symmetry of a female are independent of her own homozygosity. However, female size and the time at which females oviposit have strong effects on their number of eggs. It is thus not surprising that homozygosity could only account for a small fraction of the variation.

It can be difficult to separate fitness effects of mother and offspring. In this system the inbreeding coefficient of offspring will be very weakly correlated with that of their mothers' (if at all) and homozygosity does not affect the number of eggs females can lay. Therefore, the fact that mothers' homozygosity affects the number of offspring that reach maturity, suggest that maternal effects and not offspring genotype or number of eggs, plays a role in determining if an egg will develop into a mature wasp. Maternal factors could be the quality of the egg, or the gall from which the developing wasp larvae feed. The

potential role of gall quality was recently illustrated by two studies on alternative male morphs in non-pollinating fig wasps that showed that gall size (Moore *et al.*, 2004) and quality (Pereira *et al.*, 2007), determine males' size and morphology.

We could not confirm the correlation between F and the proportion of homozygous loci by using pedigrees. However, we showed that *P. awekei*'s mixed mating system should result in a high variance in F , which means that the multi-locus homozygosity is a good proxy for F (Pemberton, 2004).

We found evidence that outbreeding depression occurs in this fig wasp, but possibly also inbreeding depression. Despite purging of partially recessive deleterious alleles in haploid males, it is not unusual to observe some inbreeding depression in haplodiploids (Antolin, 1999; Henter, 2003). Inbreeding depression could be the result of overdominance or female limited expression (Antolin, 1999). On the other hand, if model 2 is correct and there is no inbreeding depression, this could be explained by the fact that the wasps regularly sibmate (78% of the time) and should be adapted to high levels of inbreeding. Several authors have suggested that this may be an important factor leading to no inbreeding depression (Biémont and Bouletreau, 1980; Peer and Taborsky, 2005; Dolgin *et al.*, 2007). However, more data will be required to distinguish between these two statistical models.

In line with Clarke *et al.* (1992) who studied another haplodiploid, the honeybee, we found no increase in fluctuating asymmetry as the inbreeding coefficient increased. This contrasts with a diploid species where fluctuating asymmetry was found (Neff, 2004), giving further support to the suggestion of Clarke *et al.* (1992) that the level of inbreeding may not affect developmental stability in haplodiploids.

The break up of co-adapted gene complexes is the most common reason given for outbreeding depression (Lynch and Walsh, 1998). For co-adapted gene complexes to build up, lineages need to be isolated from one another. Geographical population subdivision is a common cause of isolation and outbreeding depression is frequently found when individuals from geographically distinct populations are crossed (Pinto *et al.*, 1991; de Meester, 1993; Sorati *et al.*, 1996; Aspi, 2000; Velando *et al.*, 2006; Dolgin *et al.*, 2007). Inbreeding can also result in the isolation of lineages from one another and this may allow for the build up of different gene complexes in one population (Luna and Hawkins, 2004; Peer and Taborsky, 2005). The within population outbreeding depression recorded in this study, as well as that of Peer and Taborsky (2005), supports this view, whereas Luna and Hawkins (2004) did not find within population outbreeding depression in the wasp *Nasonia vitripennis*. It is also important to remember that co-adaptation of gene complexes can occur in one generation from extant variation and does not necessarily need many generations of new mutations and co-evolution (Templeton, 1979). The fact that our population's outbred component should contain many females that have been outbred for only one generation, supports the growing number of papers that show outbreeding depression in the F_1 generation (Peer and Taborsky, 2005; Dolgin *et al.*, 2007).

An alternative explanation for outbreeding depression could be if wasps harbour one or multiple strains of *Wolbachia* that may induce cytoplasm incompatibility (CI;

Cook and Butcher, 1999). CI causes a slight reduction in fecundity of matings between infected males and uninfected females (Cook and Butcher, 1999). In the pollinating fig wasp, *Pleistodontes imperialis*, Haine *et al.* (2006) found different strains of *Wolbachia* in three cryptic species. This suggests that *Wolbachia* may lead to reproductive isolation in pollinating fig wasps (Haine *et al.*, 2006). *Platyscapa awekei* are infected with *Wolbachia* (OFC Greyvenstein, CJ Erasmus and JMG, unpublished data) and CI may thus be the cause of outbreeding depression observed in *P. awekei* in this study. If CI occurs in this wasp, note that the male's dispersal behaviour will allow CI strains to spread faster by spoiling the reproductive success of uninfected females.

Another common explanation of outbreeding depression is a mismatch between environment and the genotypes. In these tiny wasps, however, females move over vast distances so that populations 500 km apart are genetically indistinguishable (Jansen van Vuuren *et al.*, 2006; CJ Erasmus and JMG, unpublished data), even when correcting for the highly expected heterozygosity (Hedrick, 2005). In addition, fig trees regulate the temperature inside figs to reduce the environmental variability the wasps experience (Patiño *et al.*, 1994). This means that local adaptation is very unlikely to explain outbreeding depression in *P. awekei*.

Our finding of outbreeding depression is surprising—this is a wasp that actively disperses to engage in outbreeding, which will result in the lowest fitness. According to the second statistical model where there is only outbreeding depression, both outbreeding depression, as well as the kin selective advantages, should select for a completely inbred population. This prediction is clearly at odds with the data. If the first statistical model is correct, *P. awekei* females will attain the highest fitness during the third, fourth and fifth cycles of sibmating. In this scenario a female can either sibmate and have offspring that are more inbred than she is, or she can reset the clock by mating with an unrelated male. However, a certain amount of 'frustration' is inevitable in such a mixed mating system as lineages will involuntarily wander into areas where the fitness is below the maximum. Fitness can be improved if females can respond facultatively to their own inbredness, a possibility that is not inconceivable since even borage (Drayner, 1956) and field beans (Crowe, 1971) manage to do so.

When we parameterize an unpublished model (RM Nelson and JMG) designed for a system where fitness depends on the inbredness, it predicts an optimal sibmating rate that is higher than that observed. Thus for both statistical models, these wasps outbreed too much. The suggestion of Moore *et al.* (2006) that male dispersal serves to reduce competition between related brothers seems to be a more plausible explanation than that male dispersal evolved to achieve an optimal, yet 'frustrated', mating system.

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References

- Antolin MF (1999). A genetic perspective on mating systems and sex ratios of parasitoids. *Res Popul Ecol* **41**: 29–37.
- Aspi J (2000). Inbreeding and outbreeding depression in male courtship song characters in *Drosophila montana*. *Heredity* **84**: 273–282.
- Balloux F, Amos W, Coulson T (2004). Does heterozygosity estimate inbreeding in real populations? *Molec Ecol* **13**: 3021–3031.
- Bateson P (1983). Optimal outbreeding. In: Bateson P (ed). *Mate Choice*. Cambridge University Press: Cambridge, pp 227–257.
- Bengtsson BO (1978). Avoiding inbreeding: at what cost? *J Theor Biol* **73**: 439–444.
- Biémont C, Bouletreau M (1980). Hybridization and inbreeding effects on genome coadaptation in a haplo-diploid hymenoptera: *Cothonaspis boulandi* (Eucolidae). *Experientia* **36**: 45–47.
- Bittles AH (2001). Consanguinity and its relevance to clinical genetics. *Clin Genet* **60**: 89–98.
- Brückner D (1978). Why are there inbreeding effects in haplo-diploid systems? *Evolution* **32**: 456–458.
- Campbell RB (1986). The interdependence of mating structure and inbreeding depression. *Theor Pop Biol* **30**: 232–244.
- Charlesworth D, Charlesworth B (1987). Inbreeding depression and its evolutionary consequences. *Ann Rev Ecol Syst* **18**: 237–268.
- Charlesworth D, Charlesworth B (1990). Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. *Evolution* **44**: 870–888.
- Charlesworth D, Morgan MT, Charlesworth B (1990). Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* **44**: 1469–1489.
- Clarke GM, Oldroyd BP, Hunt P (1992). The genetic basis of developmental stability in *Apis mellifera*: heterozygosity versus genic balance. *Evolution* **46**: 753–762.
- Cook JM (1993). Sex determination in the Hymenoptera: a review of models and evidence. *Heredity* **71**: 421–435.
- Cook JM, Butcher RDJ (1999). The transmission and effects of *Wolbachia* bacteria in parasitoids. *Res Popul Ecol* **41**: 15–28.
- Cowan DP (1979). Sibling matings in a hunting wasp: adaptive inbreeding? *Science* **205**: 1403–1405.
- Crowe LK (1971). The polygenic control of outbreeding in *Borago officinalis*. *Heredity* **27**: 111–118.
- de Meester L (1993). Inbreeding and outbreeding depression in *Daphnia*. *Oecologia* **96**: 80–84.
- Dolgin ES, Charlesworth B, Baird SE, Cutter AD (2007). Inbreeding and outbreeding depression in *Caenorhabditis* nematodes. *Evolution* **61**: 1339–1352.
- Drayner JM (1956). Regulation of outbreeding in field beans (*Vicia faba*). *Nature* **177**: 489–490.
- Estoup A, Largiadier CR, Perrot CR, Chourrot D (1996). Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molec Mar Biol Biotech* **5**: 295–298.
- Fisher RA (1941). Average excess and average effect of a gene substitution. *Ann Eugen* **11**: 53–63.
- Frank SA (1985). Are mating and mate competition by the fig wasp *Pegoscopus assuetus* (Agaonidae) random within a fig? *Biotropica* **17**: 170–172.
- Godfray HCJ, Cook JM (1997). Mating systems of parasitoid wasps. In: Choe JC, Crespi BJ (eds). *The Evolution of Mating Systems in Insects and Arachnids*. Cambridge University Press: Cambridge, pp 211–225.
- Goodwillie C, Kalisz S, Eckert CG (2005). The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations and empirical evidence. *Ann Rev Ecol Syst* **36**: 47–79.

- Greeff JM (2002). Mating system and sex ratios of a pollinating fig wasp with dispersing males. *Proc R Soc Lond B* **269**: 2317–2323.
- Greeff JM, van Noort S, Rasplus J-Y, Kjellberg F (2003). Dispersal and fighting in male pollinating fig wasps. *C R Biologies* **326**: 121–130.
- Greenwood PJ (1980). Mating systems, philopatry and dispersal in birds and mammals. *Anim Behav* **28**: 1140–1162.
- Haine ER, Martin J, Cook JM (2006). Deep mtDNA divergences indicate cryptic species in a fig-pollinating wasp. *BMC Evol Biol* **6**: 83.
- Hamilton WD (1967). Extraordinary sex ratios. *Science* **156**: 477–488.
- Hamilton WD (1979). Wingless and fighting males in fig wasps and other insects. In: Blum ms, Blum NA (eds). *Sexual selection and Reproductive Competition in Insects*. Academic Press: New York, pp 167–220.
- Hansson B, Westerberg L (2002). On the correlation between heterozygosity and fitness in natural populations. *Molec Ecol* **11**: 2467–2474.
- Hardy ICW (1994). Sex ratio and mating structure in the parasitoid Hymenoptera. *Oikos* **69**: 3–20.
- Hedrick PW (2005). A standardized genetic differentiation measure. *Evolution* **59**: 1633–1638.
- Helgason A, Pálsson S, Gudbjartsson DF, Kristjánsson T, Stefánsson K (2008). An association between kinship and fertility of human couples. *Science* **319**: 813–816.
- Henter HJ (2003). Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. *Evolution* **57**: 1793–1803.
- Herre EA, West SA, Cook JM, Compton SG, Kjellberg F (1997). Fig-associated wasps: pollinators and parasites, sex-ratio adjustment and male polymorphism, population structure and its consequences. In: Choe JC, Crespi BJ (eds). *The Evolution of Mating Systems in Insects and Arachnids*. Cambridge University Press: Cambridge, pp 226–239.
- Holsinger KE, Feldman MW, Christiansen FB (1984). The evolution of self-fertilization in plants: a population genetic model. *Am Nat* **124**: 446–453.
- Jain SK (1976). The evolution of inbreeding in plants. *Ann Rev Ecol Syst* **7**: 469–495.
- Jansen van Vuuren GJ, Kryger P, Greeff JM (2006). Isolation of six microsatellite loci in the pollinating fig wasp, *Platyscapa awekei*. *Molec Ecol Notes* **6**: 385–386.
- Kathuria P, Greeff JM, Compton SG, Ganeshaiah KN (1999). What fig wasp sex ratios may or may not tell us about sex allocation strategies. *Oikos* **87**: 520–530.
- Kjellberg F, Jousset E, Hossaert-McKey M, Rasplus J-Y (2005). Biology, ecology and evolution of fig-pollinating wasps (Chalcidoidea, Agaonidae). In: Raman A, Schaefer CW, Withers TM (eds). *Biology, Ecology, and Evolution of Gall-inducing Arthropods*. Science publishers Inc.: Enfield (NH) USA, Plymouth, pp 539–572.
- Kokko H, Ots I (2006). When not to avoid inbreeding. *Evolution* **60**: 467–475.
- Lande R, Schemske DW (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**: 24–40.
- LeBas NR (2002). Mate choice, genetic incompatibility, and outbreeding in the ornate dragon lizard, *Ctenophorus ornatus*. *Evolution* **56**: 371–377.
- Lloyd DG (1979). Some reproductive factors affecting the selection of self-fertilization in plants. *Am Nat* **133**: 67–79.
- Luna MG, Hawkins BA (2004). Effects of inbreeding versus outbreeding in *Nasonia vitripennis* (Hymenoptera: Pteromalidae). *Env Entom* **33**: 765–775.
- Lynch M, Walsh B (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Associates Inc.: Sunderland.
- Marr AB, Keller LF, Arcese P (2002). Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). *Evolution* **56**: 131–142.
- Marshall TC, Spalton JA (2000). Simultaneous inbreeding and outbreeding depression in reintroduced Arabian oryx. *Anim Cons* **3**: 241–248.
- Maynard Smith J (1977). Sex and evolution: the sex habit in plants and animals. In: Christiansen FB, Fenchel TM (eds). *Measuring Selection in Natural Populations*. Springer-Verlag: Heidelberg, pp 315–331.
- Maynard Smith J (1978). *The Evolution of Sex*. Cambridge University Press: Cambridge.
- Molbo D, Krieger MJB, Herre EA, Keller L (2002). Species-diagnostic microsatellite loci for the fig wasp genus *Pegoscapus*. *Molec Ecol Notes* **2**: 440–442.
- Molbo D, Machado CA, Herre EA, Keller L (2004). Inbreeding and population structure in two cryptic fig wasp species. *Molec Ecol* **13**: 1613–1623.
- Moore JC, Dunn AM, Compton SG, Hatcher MJ (2003). Foundress re-emergence and fig permeability in fig tree-wasp mutualisms. *J Evol Biol* **16**: 1186–1195.
- Moore CJ, Pienaar J, Greeff JM (2004). Male morphological variation and the determinants of body size in two Otiteselline fig wasps. *Behav Ecol* **15**: 735–741.
- Moore JC, Loggenberg A, Greeff JM (2006). Kin competition promotes dispersal in a male pollinating fig wasp. *Biol Lett* **2**: 17–19.
- Møller AP, Swaddle JP (1997). *Asymmetry, Developmental Stability, and Evolution*. Oxford University Press: Oxford.
- Neff BD (2004). Stabilizing selection on genomic divergence in a wild fish population. *Proc Natl Acad Sci* **101**: 2381–2385.
- Normark BB (2004). Haplodiploidy as an outcome of coevolution between male-killing cytoplasmic elements and their hosts. *Evolution* **58**: 790–798.
- Parker GA (1979). Sexual selection and sexual conflict. In: Blum ms, Blum NA (eds). *Sexual selection and Reproductive competition in Insects*. Academic Press: New York, pp 123–166.
- Patiño S, Herre EA, Tyree MT (1994). Physiological determinants of *Ficus* fruit temperature and implications for survival of pollinator wasp species: comparative physiology through an energy budget approach. *Oecologia* **100**: 13–20.
- Peer K, Taborsky M (2005). Outbreeding depression, but no inbreeding depression in haplodiploid ambrosia beetles with regular sibling mating. *Evolution* **59**: 317–323.
- Pemberton J (2004). Measuring inbreeding depression in the wild: the old ways are the best. *Trends Ecol Evol* **19**: 613–615.
- Pereira RA, Teixeira SP, Kjellberg F (2007). An inquiline fig wasp using seeds as a resource for small male production: a potential first step for the evolution of new feeding habits? *Biol J Linn Soc* **92**: 9–17.
- Pinto JD, Stouthamer R, Platner GR, Oatman ER (1991). Variation in reproductive compatibility in *Trichogramma* and its taxonomic significance (Hymenoptera: Trichogrammatidae). *Ann Ent Soc Am* **84**: 37–46.
- Price MV, Waser NM (1979). Pollen dispersal and optimal out-crossing in *Delphinium nelsoni*. *Nature* **277**: 294–297.
- R Development Core Team (2007). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Vienna, Austria.
- Schemske DW, Lande R (1985). The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* **39**: 41–52.
- Schierup MH, Christiansen FB (1996). Inbreeding depression and outbreeding depression in plants. *Heredity* **77**: 461–468.
- Slate J, David P, Dodds KG, Veenliet BA, Glass BC, Broad TE et al. (2004). Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* **93**: 255–265.
- Sorati M, Newman M, Hoffmann AA (1996). Inbreeding and incompatibility in *Trichogramma* nr. *brassicae*: evidence and implications for quality control. *Ent Exp Appl* **78**: 283–290.

- Stubblefield JW, Seger J (1990). Local mate competition with variable fecundity: dependence of offspring sex ratios on information utilization and mode of male production. *Behav Ecol* **1**: 68–80.
- Suzuki Y, Iwasa Y (1980). A sex ratio theory for gregarious parasitoids. *Res Popul Ecol* **22**: 366–382.
- Taylor PD, Getz WM (1994). An inclusive fitness model for the evolutionary advantage of sibmating. *Evol Ecol* **8**: 61–69.
- Templeton AR (1979). The unit of selection in *Drosophila mercatorum*. II. Genetic revolution and the origin of coadapted genomes in parthenogenetic strains. *Genetics* **92**: 1265–1282.
- Uyenoyama MK (1986). Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. *Evolution* **40**: 388–404.
- Uyenoyama MK, Waller DM (1991a). Coevolution of self-fertilization and inbreeding depression I. Mutation-selection balance at one and two loci. *Theor Pop Biol* **40**: 14–46.
- Uyenoyama MK, Waller DM (1991b). Coevolution of self-fertilization and inbreeding depression II. Symmetric overdominance in viability. *Theor Pop Biol* **40**: 47–77.
- Uyenoyama MK, Holsinger KE, Waller DM (1993). Ecological and genetic factors directing the evolution of self-fertilization. *Oxf Surv Evol Biol* **9**: 327–381.
- Velando A, Domínguez J, Ferreiro A (2006). Inbreeding and outbreeding reduces cocoon production in the earthworm *Eisenia andrei*. *Eur J Soil Biol* **42**: S354–S357.
- Waddington KD (1983). Pollen flow and optimal outcrossing distance. *Am Nat* **122**: 147–151.
- Werren JH (1993). The evolution of inbreeding in haplodiploid organisms. In: Thornhill NW (ed). *The Natural History of Inbreeding and Outbreeding*. The University of Chicago Press: Chicago, pp 97–117.
- Zavadna M, Arens P, Vosman B, van Dijk PJ, van Damme JMM (2002). Development and characterization of microsatellite markers for *Liporhophalum tentacularis* Grandi, the pollinator fig wasp of *Ficus motana* Blume. *Molec Ecol Notes* **2**: 569–571.
- Zavadna M, Compton SG, Biere A, Gilmartin PM, van Damme JMM (2005). Putting your sons in the right place: the spatial distribution of fig wasp offspring inside figs. *Ecol Entomol* **30**: 210–219.