

ORIGINAL ARTICLE

Male offspring production by asexual *Potamopyrgus antipodarum*, a New Zealand snailM Neiman¹, K Larkin¹, AR Thompson^{1,2} and P Wilton^{1,3}

As only females contribute directly to population growth, sexual females investing equally in sons and daughters experience a two-fold cost relative to asexuals producing only daughters. Typically, researchers have focused on benefits of sex that can counter this 'cost of males' and thus explain its predominance. Here, we instead ask whether asexuals might also pay a cost of males by quantifying the rate of son production in 45 experimental populations ('lineages') founded by obligately asexual female *Potamopyrgus antipodarum*. This New Zealand snail is a powerful model for studying sex because phenotypically similar sexual and asexual forms often coexist, allowing direct comparisons between sexuals and asexuals. After 2 years of culture, 23 of the 45 lineages had produced males, demonstrating that asexual *P. antipodarum* can make sons. We used maximum-likelihood analysis of a model of male production in which only some lineages can produce males to estimate that ~50% of lineages have the ability to produce males and that ~5% of the offspring of male-producing lineages are male. Lineages producing males in the first year of the experiment were more likely to make males in the second, suggesting that some asexual lineages might pay a cost of males relative to other asexual lineages. Finally, we used a simple deterministic model of population dynamics to evaluate how male production affects the rate of invasion of an asexual lineage into a sexual population, and found that the estimated rate of male production by asexual *P. antipodarum* is too low to influence invasion dynamics.

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INTRODUCTION

All-female lineages have long fascinated evolutionary biologists, largely because the absence of males implies persistently asexual reproduction. These lineages also highlight what has been termed the 'queen of problems' (Bell, 1982) in evolutionary biology: the overwhelming predominance of sex despite the frequent production and persistence of successful asexual lineages (Maynard Smith, 1971, 1978; Williams, 1975). This apparent paradox stems from the fact that only females can produce offspring, which means that only females can directly contribute to the rate of population growth. The implications are that an asexual, all-female lineage that invests entirely in daughters will rapidly replace an otherwise identical sexual population that allocates resources equally to the production of sons and daughters (Maynard Smith, 1971, 1978; Lively, 1996).

For dioecious taxa, the reproductive advantage generated by the asexual production of offspring is only realized in full if asexual females do not invest in sons. However, males have been discovered in populations of a diverse array of animal lineages that are otherwise all female and presumably obligately asexual (Hebert *et al.*, 1989; Browne, 1992; Butlin *et al.*, 1998; Smith *et al.*, 2006; Lunt, 2008), suggesting that occasional male production might characterize many different asexual taxa. Production of sons by asexual females is of interest because it could reduce the otherwise two-fold cost of sex experienced by sexuals that coexist with asexual individuals as well

as lower the fitness of male-producing asexual females relative to coexisting asexual females that only make daughters (Innes *et al.*, 2000).

Potamopyrgus antipodarum, a New Zealand freshwater prosobranch snail, is characterized by the frequent coexistence of phenotypically and ecologically similar sexual males and females with what are presumed to be obligately asexual females (Lively, 1987, 1992; Jokela *et al.*, 1997). Both nuclear (Dybdahl and Lively, 1995) and mitochondrial data (Neiman and Lively, 2004; Neiman *et al.*, 2011) demonstrate that asexuals originated via multiple independent and usually recent transitions from sexual *P. antipodarum*. These characteristics of *P. antipodarum* enable direct and powerful comparisons between sexual and asexual forms and among populations that vary in the relative frequency of sexual individuals (Maynard Smith, 1978; Jokela *et al.*, 1997; Lively, 1992). The recent sexual ancestry of asexual *P. antipodarum* also means that they are likely to retain traits inherited from their sexual ancestors, such as copulatory behavior (Neiman, 2004; Neiman and Lively, 2005; Nelson and Neiman, 2011) and, perhaps, the ability to produce sons (Neiman *et al.*, 2011).

As asexual *P. antipodarum*, like many asexual animals (Suomalainen, 1987; Otto and Whitton, 2000), are polyploid (triploid or higher; Neiman *et al.*, 2011; Liu *et al.*, 2012) whereas sexuals are diploid (Wallace, 1992), the occasional documentation of polyploid male *P. antipodarum* within lake populations that are otherwise

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dominated by female asexuals (Wallace, 1992; Lively and Jokela, 2002; Neiman *et al.*, 2011) hinted that at least some asexual *P. antipodarum* might pay a cost of males. Here, we addressed this possibility by evaluating whether and how often experimental populations founded by a diverse set of female asexual *P. antipodarum* collected from multiple different New Zealand lake populations produce males and whether some asexual lineages are more likely to produce males than others.

MATERIALS AND METHODS

Isolation and culture

In the spring of 2009, we initiated asexual lineages by isolating adult (>3 mm in shell length; Jokela and Lively, 1995) female *P. antipodarum* that had been collected from each of 24 New Zealand lake populations earlier that year. We isolated 5–15 females per lake sample, depending upon the number of adult females available for each sample and whether adult females from that particular collection were needed for other studies. Each female was marked with nail polish to enable future identification and was housed alone in an one-liter clear plastic cup. We surveyed each cup every 4 weeks to determine whether each female had produced offspring. As some asexual *P. antipodarum* lineages do not thrive in laboratory conditions (Jokela *et al.*, 2003), and as some females may have been parasitically castrated (Lively, 1992), we removed females that had not produced any offspring within this or any previous 4-week period and replaced them with another adult female from the same field collection. Each set of adult females and their offspring were transferred together to a separate 51 plastic tank once 10 offspring had appeared in the cup, such that each tank contained a founding female and all of the offspring she had produced while still in the cup. We continued to maintain each tank (each containing a single, independently-derived asexual lineage descended from a single founding female) for a total of 2 years after the isolation of the founding female.

Ploidy determination

We randomly selected between 1–5 individuals from each lineage to use for determination of ploidy level. Following Neiman *et al.* (2011), we prepared each sample for flow cytometry by first dissecting the head tissue away from the body and then grinding head tissue in a solution containing 0.2 M Tris-HCl (pH 7.5), 4 mM MgCl₂, 1% TritonX-100, and 4 µg ml⁻¹ DAPI. This solution was filtered through a 70-micron nylon sheet and then run on a Beckman-Coulter Quanta SC MPL flow cytometer (Indianapolis, IN, USA). We used the FL1 channel to assess the DAPI fluorescence (and thus the DNA content) of sample nuclei under a UV lamp. At the beginning of each flow cytometry run, we calibrated the machine with 20 µl of chicken red blood cells (Lampire Biological Labs, Pipersville, PA, USA) treated and filtered as for the snail head tissue. We adjusted the gain so that the chicken standard peak was always centered on 200 FL1 units. Each sample was run until a count of 10 000 events was achieved.

Sex ratio determination

In the spring of 2010, after 1 year of culture (1–2 generations for *P. antipodarum*; Dybdahl and Kane, 2005), we sexed all individuals in each tank that were large enough (>3 mm shell length) to have reached sexual maturity (Jokela and Lively, 1995). Adult male *P. antipodarum* are distinguished from females by the presence of a penis on the right side of the back of the head (Winterbourn, 1970a). We recorded the number of all the adult males and adult females (minus the original female, if still present) found in each tank at this time (hereafter, '2010 males' and '2010 females'). Each of the 2010 males received a permanent mark on the back of his shell with nail polish to avoid counting them as newly-produced males in the next culture period. We repeated this sexing procedure in the spring of 2011 after 2 years of culture; as *P. antipodarum* become sexually mature in 6–9 months (Dybdahl and Kane, 2005), 2 years of culture is equivalent to 3–4 generations.

All males produced since the 2010 census are referred to as '2011 males'. As the number of female snails produced in many tanks was so high (>500) that marking individual females was prohibitive, we calculated the number of new

females produced since the 2010 census ('2011 females') by subtracting the number of females counted in 2010 from the number of females counted in 2011. Any tank for which this value was negative was recorded as producing zero 2011 females.

Data analysis

Flow cytometry. We followed very closely the procedure for flow cytometry used in Neiman *et al.* (2011). Briefly, we first used the Beckman Quanta Analysis software to confine data analysis for each flow cytometry sample to the peak of data points corresponding to intact nuclei of single cells in growth phase 1. We calculated the mean fluorescence for this peak region for each sample and standardized this mean by dividing it by the mean FL1 value of the chicken red blood cell standard used to calibrate that particular run. Next, we divided this value by the mean FL1 value of a *P. antipodarum* haploid genome (as determined in the 2009 data set in Neiman *et al.*, 2011) to determine how many haploid genome equivalents were represented by the sample. As in Neiman *et al.* (2011), individuals with nuclear genome contents $\leq 2.39 \times$ were designated as diploid, individuals with nuclear genome contents $> 2.39 \times$ but $\leq 3.5 \times$ were classified as triploid, and individuals with nuclear genome contents $> 3.5 \times$ were classified as having a ploidy level exceeding triploid (' $> 3 \times$ '). We tested the repeatability of the flow cytometry procedure by measuring the fluorescence of individual *P. antipodarum* samples twice during the course of a single flow cytometry run. The two measures were strongly and positively correlated ($N = 12$ individuals, $r = 0.97$, $P < 0.0001$).

Comparisons of rate and prevalence of son production across ploidy levels and culture periods. For each of the two 1-year culture periods (2009–2010; 2010–2011), we counted the number of new males and the total number of newly produced offspring. The absence of male offspring can indicate either that a lineage is incapable of producing sons or that it is capable of making sons but by chance did not. For this reason, we estimated the frequency of male production amongst lineages in two ways. First, we quantified prevalence of male-producing lineages and the rate of male production amongst male producers, classifying lineages as male producing or non-male producing based on the presence or absence of males. In the absence of more statistically tractable alternatives, we used these 'naive' classifications for comparisons of male production between years and across ploidy levels. Second, to refine our estimate of the prevalence and rate of male production by asexual females, we carried out a maximum-likelihood analysis of two models that take into account the possibility that some male-producing lineages may not have produced any male offspring just by chance.

For our first (naive) estimation of male production rates and prevalence, we classified any lineage that had new males in a particular culture period as male producing for that culture period, whereas lineages that did not produce new males in a given culture period were classified as non-male producing for that culture period. Because the absence of new males in lineages that did not produce new offspring is not informative, we excluded the seven lineages that did not produce new offspring in 2010–2011 from all further analyses involving the 2010–2011 data set, leaving us with 38 lineages for those analyses. These male frequency data did not meet the normality assumptions of parametric tests, so they were analyzed non-parametrically. We used Kruskal–Wallis tests to determine whether there were significant differences in our estimates of the rate of male production between the two asexual ploidy levels ($3 \times$, $> 3 \times$) for each of the two culture periods. Based on the premise that across-lineage genetic variation for son production—if any—should also translate into across-year associations in the presence or absence of son production within lineages, we used a Fisher's exact test to determine whether lineages that had produced any males in 2009–2010 ('male producing') were also more likely than lineages that had not made any males in 2009–2010 ('non-male producing') to produce males in 2010–2011.

Maximum-likelihood estimation of prevalence and rate of male production. We carried out a maximum-likelihood analysis of two models of male production that take into account the possibility that some lineages possessing the capability to produce males did not produce any males just by chance. In the first model, all lineages are capable of producing male offspring, and each offspring is male with probability p . Under this model, the number of males m

in a group of offspring of size n is binomially distributed with convolution parameter n and probability parameter p . The likelihood $L(p|\mathbf{n},\mathbf{m})$ of p given offspring group sizes n and male counts m is then

$$L(p|\mathbf{n},\mathbf{m}) = \prod_{i=1}^l \binom{n_i}{m_i} p^{m_i} (1-p)^{n_i-m_i},$$

where l is the number of lineages.

Under the second model, each lineage is capable of producing males with probability ψ and is incapable of producing males with probability $1-\psi$. As in the first model, each offspring of a male-producing lineage is male independently with probability p . Thus, the absence of male offspring in a given group of offspring in this model indicates one of two possibilities: (1) the lineage lacks the capacity to produce male offspring, or (2), the lineage has the capacity to produce male offspring but by chance did not produce any males in this group of offspring. Under this model, the probability $P(n,m|\psi,p)$ that a group of n newly-produced offspring contains m males is

$$P(n,m|\psi,p) = \psi \binom{n}{m} p^m (1-p)^{n-m} + (1-\psi) \delta_{0,m},$$

where $\delta_{0,m}$ is Kronecker's delta. The likelihood $L(p,\psi|\mathbf{n},\mathbf{m})$ of the parameters given offspring group sizes \mathbf{n} and male counts \mathbf{m} is

$$L(p,\psi|\mathbf{n},\mathbf{m}) = \prod_{i=1}^l P(n_i, m_i | \psi, p).$$

To compare the two models and test the possibility that not all lineages are capable of producing males, we carried out a likelihood-ratio test after maximizing the likelihood functions of the two models. For the second model, a joint 95% profile confidence region was calculated for the two parameters.

RESULTS

Sex ratio and rate of male production

We established 45 lineages descended from single females that were originally sampled from 13 different New Zealand lakes (Table 1; females from 11 of the 24 lakes that we used as potential founders did not produce offspring). In all, 26 of these lineages were triploid and 19 had ploidy levels higher than triploid ($>3 \times$).

Males were found in eleven of the twenty-six triploid lineages (42.3%) and twelve of the nineteen $>3 \times$ lineages (63.2%), for a total of 23 (51.1%) of the 45 asexual lineages. The mean frequency of males produced was $2.52 \pm 5.61\%$ s.d. of offspring produced in 2009–2010 (calculated from all 45 lineages) and $12.3 \pm 30.84\%$ s.d. of offspring produced in 2010–2011 (calculated from the 38 lineages that also made offspring during this culture period). When we removed the four lineages for which 100% of new offspring produced in 2010–2011 were males but where the total number of new offspring produced in 2010–2011 was no more than four, the mean frequency of males produced in 2010–2011 was $1.98 \pm 4.94\%$ of new offspring produced. In the 51.11% of lineages that produced males, the mean frequency of males amongst new offspring was $6.68 \pm 7.57\%$ in 2009–2010 and was $31.15 \pm 43.41\%$ in 2010–2011. When the four lineages that made 100% males in 2010–2011 were excluded from this calculation, the mean male frequency amongst male-producing lineages that year was $6.11 \pm 7.27\%$. Across both years, the mean frequency of new males produced within new lineages was $2.47 \pm 4.19\%$ s.d. These data are summarized in Figure 1 and Table 1.

Although we cannot formally exclude the possibility that these lineages could have produced at least some offspring (including the males) sexually, the rate of male production by the lineages in our study was at least $5\text{--}10 \times$ lower than that recorded from females sampled from highly sexual *P. antipodarum* populations (Wallace, 1992). Thus, the extreme female-biased sex ratios of all lineages—male producing or not—suggests that they are asexual.

Table 1 New Zealand lake sources, ploidy levels, and rate and presence of son production for 45 asexual *P. antipodarum* lineages

Lake (no. of lineages)	$3 \times$	$>3 \times$	% 2009–	% 2010–	% new males	Male production	
			2010 males	2011 males	(no. of new snails)		
			(no. of new snails)	(no. of new snails)			
			2009–2010	2010–2011			
Clearwater (2)	X		0 (44)	0 (20)	0 (64)	N	
	X		0 (45)	0 (69)	0 (114)	N	
Grasmere (1)	X		5 (18)	0.6 (168)	1.1 (186)	Y	
	X		0 (49)	0 (1)	0 (50)	N	
Gunn (13)	X		0 (18)	100 (4)	18.18 (22)	Y	
		X	0 (63)	0 (0)	0 (63)	N	
		X	0 (36)	0 (26)	0 (62)	N	
		X	0 (19)	0 (78)	0 (97)	N	
		X	0 (13)	7.5 (40)	5.66 (53)	Y	
		X	0 (21)	4.8 (126)	4.08 (147)	Y	
		X	1.4 (72)	0 (0)	1.39 (72)	Y	
		X	3.1 (32)	100 (1)	6.06 (33)	Y	
		X	3.8 (53)	0 (12)	1.54 (65)	Y	
		X	4.3 (23)	0 (28)	1.96 (51)	Y	
		X	4.3 (23)	16.7 (12)	8.57 (35)	Y	
		X	6.3 (16)	5.2 (58)	5.41 (74)	Y	
	Heron (3)	X		0 (22)	0 (0)	0 (22)	N
X			0 (9)	0 (38)	0 (47)	N	
X			2.5 (40)	0 (12)	1.92 (52)	Y	
Kaniere (1)	X		33 (6)	0 (43)	4.08 (49)	Y	
	X		0 (59)	0 (51)	0 (110)	N	
Okareka (4)	X		0 (8)	0 (103)	0 (111)	N	
	X		8 (50)	0 (0)	8 (50)	Y	
	X		9.9 (81)	0 (0)	9.88 (81)	Y	
	X		0 (78)	0 (6)	0 (84)	N	
Poerua (3)	X		2.3 (44)	22.2 (9)	5.66 (53)	Y	
	X		4.2 (142)	100 (1)	4.9 (143)	Y	
	X		0 (43)	0 (70)	0 (113)	N	
Rotoroiti (9)	X		0 (9)	0 (32)	0 (41)	N	
	X		0 (44)	0 (57)	0 (101)	N	
	X		0 (34)	0 (77)	0 (111)	N	
	X		0 (38)	0 (615)	0 (653)	N	
	X		0 (12)	0 (250)	0 (262)	N	
	X		0 (31)	0.3 (375)	0.25 (406)	Y	
	X		0 (28)	0.8 (713)	0.81 (741)	Y	
	X		8.3 (12)	0.7 (678)	0.87 (690)	Y	
	Rotoroa (2)	X		0 (2)	0 (651)	0 (653)	N
		X		0 (33)	7.7 (26)	3.39 (59)	Y
Sarah (1)	X		0 (51)	0 (0)	0 (51)	N	
Taupo (1)	X		0 (21)	0 (49)	0 (70)	N	
Taylor (1)	X		1.1 (87)	0.7 (135)	0.9 (222)	Y	
Waikaremoana (4)	X		0 (66)	0 (76)	0 (142)	N	
	X		0 (83)	0 (0)	0 (83)	N	
	X		2.2 (44)	0 (265)	0.32 (309)	Y	
	X		13.9 (137)	100 (4)	16.3 (141)	Y	

The seven lineages that did not produce any offspring in the second (2010–2011) culture period were excluded from any analyses that included the 2010–2011 data set (gray shading).

To obtain more rigorous estimates of the probability that an offspring of a male-producing lineage is male and the probability that a lineage is a male producer, we performed maximum-likelihood analysis of two models of male production. A likelihood ratio test showed that the model in which all lineages are capable of producing males and produce males at the same rate can be rejected (likelihood

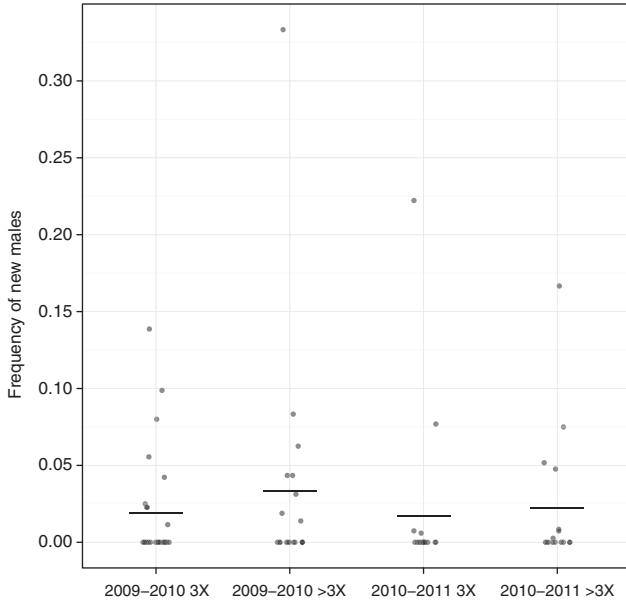


Figure 1 Jitterplot showing the mean percent of new males (horizontal bars; relative to total new offspring) produced in the first (2009–2010) and second (2010–2011) years of culture for $3\times$ and $>3\times$ lineages. All 45 lineages are represented for 2009–2010. For 2010–2011, we excluded the seven lineages that made no offspring as well as the four lineages for which the new male frequency was 100% but where the total number of new offspring was four or less. There was not a significant difference in the frequency of males produced between the different ploidy levels for either culture period.

ratio = 279.8, degree of freedom = 1, $P < 10^{-16}$). Under an alternative model in which some lineages are capable of producing males and others are not, the maximum-likelihood estimate of the probability of being a male producer, ψ , was 0.482 (bounds of 95% profile likelihood joint confidence region: 0.281–0.703), and the maximum-likelihood estimate of P , the probability that each offspring of a male producer is male, was 0.053 (bounds of 95% profile likelihood joint confidence region: 0.03584–0.0749).

Using the naive estimates of male-production rates, we found no significant difference between $3\times$ and $>3\times$ lineages in the apparent frequency of males produced within either culture period (Kruskal–Wallis, 2009–2010: $P = 0.618$; 2010–2011: $P = 0.389$). The outcome of this analysis was the same when the four lineages that produced 100% males in 2010–2011 were excluded from this analysis (Kruskal–Wallis, $P = 0.18$) (Figure 1).

Lineages that were male producing in culture period 1 were more likely than non-male producing lineages to also produce males in culture period 2 (Fisher's exact test, $P = 0.037$; Figure 2). However, this result must be viewed with caution, as some apparently non-male-producing lineages produced so few offspring in 2010–2011 that the lack of males might simply reflect the low probability of son presence when only a few offspring are produced.

How does the production of males affect time to fixation for an invading asexual lineage?

We developed a deterministic model of lineage growth as a function of male production to understand how the observed rates of son production by asexual *P. antipodarum* might influence the time dynamics of an asexual lineage invading an established sexual population.

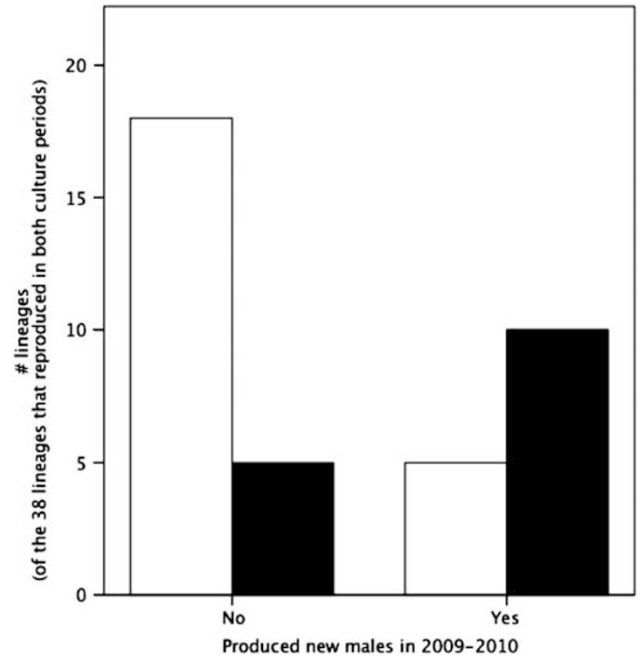


Figure 2 The number of male producing vs non-male producing lineages in 2009–2010 distinguished by whether they were male producing in 2010–2011. The seven lineages that did not produce any offspring in 2010–2011 were excluded from this figure, leaving us with a 38-lineage dataset. Lineages that made males in 2010–2011 (black) were significantly more likely to have made males in 2009–2010 than lineages that did not make males in 2010–2011 (white), though the low number of offspring produced by many lineages in 2010–2011 means that we cannot ascribe 'non-male producing' status to lineages in 2010–2011 with high confidence.

This model considers a population of females of size N . Females can reproduce either sexually ('sexual females') or asexually ('asexual females'). Let the number of asexual females at time t be represented by n_t , so that $N - n_t$ is the number of sexual females. Let k_a represent the proportion of an asexual female's offspring that is female (where proportion $1 - k_a$ is male), and k_s represent the proportion of a sexual female's offspring that is female. We assume that sexual females are never male limited. Let w_a and w_s represent the fecundities of asexual and sexual females, respectively. If p_t represents the proportion of females in the population that are asexual at time t , then

$$p_{t+1} = \frac{n_t w_a k_a}{(N - n_t) w_s k_s + n_t w_a k_a} = \frac{p_t w_a k_a}{(1 - p_t) w_s k_s + p_t w_a k_a}.$$

If the sexual offspring sex ratio is 1:1 female–male ($k_s = 0.5$) and the asexual and sexual females are assumed to have the same fecundity ($w_a = w_s$), then

$$p_{t+1} = \frac{p_t k_a}{\frac{1}{2}(1 - p_t) + p_t k_a}.$$

This recurrence relation has solution

$$p_t = \frac{2^t}{2^t - \left(1 - \frac{1}{p_0}\right) \left(\frac{1}{k_a}\right)^t},$$

where p_0 is the proportion of females that are asexual at time $t = 0$.

If $P_0 = 1/N$ when a single asexual female is introduced into the population, and t_f is the time elapsed, whereas the proportion of

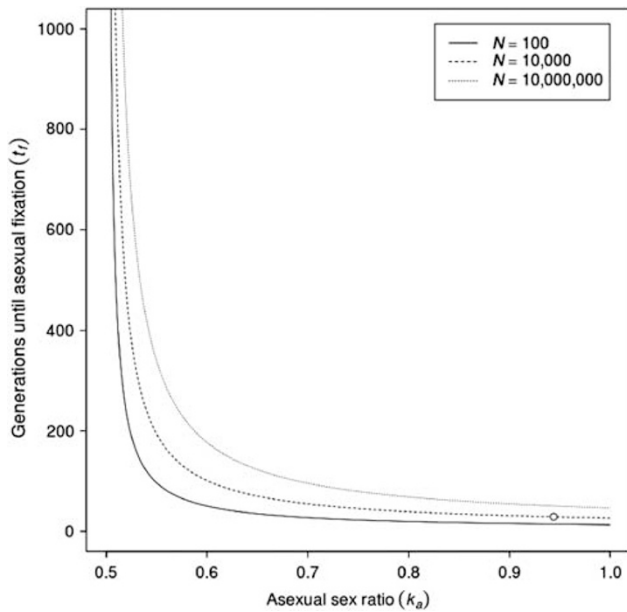


Figure 3 Generations until fixation for the descendants of a single asexual female introduced into a sexual population (t_f) as a function of proportion of asexual offspring that are female (k_a). In our model, $t_f = 2 \ln(N-1) / \ln(2k_a)$. Curves for three different population sizes (N) are shown. The maximum-likelihood estimate of k_a is plotted for reference.

females that is asexual increases from $P_0 = 1/N$ to $P_t = (N-1)/N$, then

$$t_f = \frac{2 \ln(N-1)}{\ln(2k_a)}.$$

Observed population densities in populations of *P. antipodarum* range from several hundred through many thousands of individuals per square meter of suitable surface (Dorgelo, 1987; Schreiber *et al.*, 1998). Although we cannot directly extrapolate these densities to actual census population sizes, they indicate that *P. antipodarum* populations number at least in the hundreds, and may approach or surpass one million. This is the basis for the range of population sizes we used to model time to fixation of a new asexual lineage in an established sexual population as k_a decreases from unity (no male production by asexual females) to 0.5. These results show that time until fixation initially increases only very slowly as asexual offspring sex ratio transitions from all female to 1:1 female–male. The values of k_a , we estimate here in *P. antipodarum* (range 0.93–0.96, see above), are well above the approximate value of 0.70 required for a substantial increase in fixation time of an asexual lineage for population sizes of order 10^2 – 10^7 (Figure 3).

DISCUSSION

We found that asexual female *P. antipodarum* regularly but infrequently produce sons, and that the frequency of son production did not differ across ploidy levels. That triploid and $>3 \times$ lineages produced males at comparable rates is consistent with a recent report of similar relative frequency of males among triploid and $>3 \times$ individuals collected from New Zealand lakes (Neiman *et al.*, 2011). The similar rate of male production in triploid and $>3 \times$ *P. antipodarum* indicates that $>3 \times$ individuals probably reproduce via a mechanism akin to the apomictic parthenogenesis already documented in triploid *P. antipodarum* (Phillips and Lambert, 1989),

and provides another line of evidence besides extreme female bias (Neiman *et al.*, 2011; data presented herein) that $>3 \times$ *P. antipodarum* are asexual.

Lineages producing sons in the first year of culture were significantly more likely to produce them in the next. This positive across-year association in son production suggests that there might be heritable variation for the propensity to produce sons among asexual *P. antipodarum*. However, this result must be viewed with caution given the low number of offspring produced by many non-male producing lineages in the second year of culture and because we cannot discriminate between one vs multiple females within a lineage being the source of sons. We also cannot exclude the possibility that other sources of across-lineage variation could influence male production.

Offspring production by female asexual *P. antipodarum* is resource limited (Dorgelo *et al.*, 1995; Neiman, 2006), with the implication that lineages that produce sons might produce fewer daughters than lineages that do not produce males. If the drop in daughter production is proportional to the number of sons produced, the 5–10% decrease in daughter production experienced by son-producing asexual females could influence the outcome of competition amongst asexual lineages. Hypothetically, a tradeoff between son and daughter production could also facilitate the maintenance of sex by reducing the fitness differential between sexual and asexual females. However, we used a simple model to show that on average, the rate of male production that we documented in asexual female *P. antipodarum* is so low that it is not likely to substantially slow the rate of invasion of established sexual populations by asexual lineages.

To what extent does documentation of male production in laboratory cultures of asexual female *P. antipodarum* permit inferences about natural populations? There are two lines of evidence that do suggest that the same phenomenon is likely to occur in nature. First, the mean frequency of son production by male-producing asexual lineages in our study— $\sim 5\%$ —is very similar in magnitude to the relative frequency of males reported from samples collected from ‘low male’ (mostly to entirely asexual) *P. antipodarum* populations in New Zealand (Winterbourn, 1970b; Wallace, 1992; Lively and Jokela, 2002; Neiman *et al.*, 2011). Second, polyploid male *P. antipodarum* collected from natural New Zealand populations have mitochondrial haplotypes typically associated with asexual females (Neiman *et al.*, 2011). Taken together, these data are consistent with an asexual female source for the polyploid male *P. antipodarum* documented in many New Zealand lake populations (Lively and Jokela, 2002; Neiman *et al.*, 2011). Aside from the potential costs of male production, these males are also of interest in terms of their potential to contribute to offspring production by coexisting females. Although this question is yet to be addressed in *P. antipodarum*, some possible consequences and implications of the existence of these polyploid males are discussed in Neiman *et al.* (2011).

How do asexual females produce male offspring?

Virtually nothing is known about sex determination in *P. antipodarum* (Wallace, 1992), and the lability of sex determination mechanisms in other prosobranch snails (Vitturi *et al.*, 1995; Thiriot-Quiévreux, 2003; Yusa, 2007) means that we can make only very limited inferences into *P. antipodarum* sex determination at this time.

One system that at least superficially resembles *P. antipodarum* in the occasional production of male offspring by otherwise all-female asexual lineages is *Artemia parthenogenetica*, the asexual brine shrimp (MacDonald and Browne, 1987; Browne, 1992). Female *Artemia* are

heterogametic, whereas males are homogametic (Stefani, 1960). Stefani (1964) argued that male *A. parthenogenetica* are generated when two X-bearing haploid cells fuse during oogenesis. If female *P. antipodarum* are also heterogametic, as are some other prosobranch snails (Baršienė *et al.*, 2000; reviewed in Yusa (2007)), a similar mechanism could explain the occasional production of male *P. antipodarum* by asexual females. Another potential explanation for male production in asexual lineages of *P. antipodarum* could apply if sex determination is similar to that characterized in another prosobranch snail family, Littorinidae, where females are XX and males are XO (Vitturi *et al.*, 1995). Here, occasional nondisjunction during oogenesis in female asexual *P. antipodarum* could generate XO eggs that develop into male individuals. Cytological studies directly aimed at characterizing sex determination in *P. antipodarum* and the karyotypes of male and female diploid and polyploid *P. antipodarum* will be required to discriminate between these and other possible explanations for male production by asexual females.

DATA ARCHIVING

Data were deposited in the Dryad repository: doi: 10.5061/dryad.q41jr576.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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