Manipulating sex ratios for conservation: short-term risks and long-term benefits

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Abstract

Manipulating family sex ratio is often possible, either through non-invasive methods like changing sex-determining ecological or social factors, or through more invasive methods such as hormone treatment of embryos or sperm sexing prior to using assisted reproductive technologies. If the number of available eggs limits population growth, the production of relatively more daughters than sons may eventually lead to increased population growth in terms of absolute numbers. However, any deviation of the effective sex ratio from equality increases the rate of inbreeding and the loss of genetic variance in the next generation. I show here that there is a range of female biased sex ratios where increased population growth outweighs the effect of an enhanced inbreeding rate during the first generation or the first few generations after the start of a sex ratio manipulation programme. This is especially so in small and declining populations, where some sex ratio manipulations not only increase the effective population number N_e, but also shift the population quickly into population numbers that are safe against the Allee effect. Consequently, an optimal sex ratio manipulation with respect to the genetic quality of a population means sending an endangered population first through a genetic bottleneck to achieve increased N_e, and hence decreased rates of inbreeding, in the long run.

INTRODUCTION

Sex ratio manipulation and conservation

Every sexually produced offspring has a father and a mother. This creates frequency-dependent selection on the production of sons and daughters and explains why equal primary sex ratios, i.e. 1:1 sex ratios at the end of parental investment, are so widespread (Fisher, 1930). Equal primary sex ratios under natural conditions are, however, a rule with exceptions. There is growing evidence in many species that parents are able to manipulate family sex ratio. Understanding of how these species accomplish manipulations may permit biologists to alter sex ratios as well. Such a manipulation is relatively straightforward in species with environmental sex determination, such as in many reptiles, but there are a number of tricks that are already used or could potentially be used to influence sex ratio even in mammals and birds.

Natural or artificially produced sex ratios are an important issue in conservation biology, because they are expected to impact the short- and long-term survival of endangered populations. There are a number of sce-narios in which manipulations of sex ratios may, over a certain time span, be a desired option to protect endangered species or populations. The most obvious ones are as follows:

1. Very small or rapidly declining population sizes of endangered species call for emergency actions like, for example, captive-breeding programmes (Gibbons, Durrant & Demarest, 1995; Ballou & Foose, 1996) or the use of assisted reproductive technology (ART; e.g., Gibbons *et al.*, 1995; Dobson & Lyles, 2000; Lanza, Dresser & Damiani, *et al.*, 2000). The increase of population size to above critical levels is one of the first aims of these programmes. As maximal population growth is restricted by the availability of oocytes rather than by the availability of sperm, manipulating sex ratio towards more females might be desirable under some circumstances.
2. The sex ratio in small and endangered populations is sometimes skewed for a variety of reasons. Manipulating the sex ratio of the coming generations towards less skewed ratios (e.g., to reduce intra-sexual conflict and thereby the risk of injury and death), or towards different types of skews, might be one of the aims of conservation efforts, especially if the present skew is male biased.

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In the following, I will indicate a number of different causes of natural and artificial sex ratio skews, and I will outline the potential for manipulating sex ratios towards equality, towards more females or towards more males. I will then use a simple model to illustrate the implications that sex ratio manipulation could have for conservation efforts. Manipulating sex ratios may be of benefit for some conservation in the long run, but there are a number of potential risks which should be considered.

Natural and artificial skews of sex ratios

Sex ratios are very easy to measure incidentally as part of another study. They therefore seem particularly prone to selective reporting, and an observed deviation from the 1:1 expectancy on the population level can sometimes be a chance event (Palmer, 2000). It is possible that some of the published generalizations about modified sex ratios may eventually turn out to be unfortunate and wrong. Nevertheless, there are good reasons why skewed sex ratios sometimes do occur.

If, for example, one sex is more costly to produce than the other, sex allocation theory would predict that the population sex ratio at equilibrium is biased in favour of the cheaper sex (Fisher, 1930; Charnov, 1982). This is so because parents are expected to weight their investment with the potential fitness return of an offspring. Sex allocation theory also predicts that parents that adjust their offspring sex ratio, in response to deviations from this equilibrium at the population level, will maximize their fitness over parents that do not. There is evidence in some species that adaptive sex ratio adjustment occurs (Perret, 1996; Seigel, Sheil & Doody, 1998), although the precise physiological mechanisms involved are often unclear.

One situation in which the relative reproductive value of sons and daughters may differ between individual females is where the fitness of offspring of one sex is more dependent on resources received from the parent than the fitness of offspring of the other sex. This situation may arise, for example, in polyandrous species where a few individuals of one sex can achieve a very high reproductive success at the expense of those members of the same sex that are out-competed for mating opportunities. In this case, parents should produce more offspring of the sex with the greater requirements when in good conditions or when resources are plentiful (Trivers & Willard, 1973). There is much evidence in support of this prediction, as sex ratio at birth or at the end of parental investment is often dependent on the mother's condition in a variety of taxa (Gomendio et al., 1990; Cassinello & Gomendio, 1996; Bradbury & Blakey, 1998; Kilner, 1998).

Apart from the potential influence of the maternal condition on family sex ratio, it has also been suggested that females should adjust the sex ratio of their offspring in response to characteristics of their mate, to maximize their own lifetime reproductive success (Williams, 1966). One prediction is that they might prefer to get more sons from more attractive males, because these sons are likely to be attractive themselves. A number of studies have found that this prediction is met: females get relatively more sons if they are mated with an attractive male than if they are mated with a less attractive one (Burley, 1981; Ellegren, Gustafsson & Sheldon, 1996; Sheldon et al., 1999); but see Westerdahl et al., 1997 and Saino, Ellegren & Møller, 1999.

There are many other possible causes for deviations from the Fisherian sex ratio, and some of these causes may not be the consequence of parental manipulation that is of direct adaptive value to either parent (Krackow, 1995). For example, if sex determination is controlled by independently segregating sex chromosomes (e.g., the *XX*/*XY* system in mammals), the sex ratio at conception may sometimes be fixed at 1:1. In such cases, maternal manipulation by selective abortion may sometimes be too costly. As a consequence, the degree of bias in the birth sex ratio may be related to the likely degree of sex differential embryo mortality (Clutton-Brock, 1991).

Other social factors have been identified which influence primary sex ratios. In lemurs, for example, urine or social stimuli seem to influence the sex ratio of a female's offspring (Perret, 1996). Sequential sex determination has been documented in birds (Bednarz & Hayden, 1991), mammals (Saltz & Rubenstein, 1995) or fish (Vincent & Sadovy, 1998). The physiological mechanisms behind these phenomena are often unclear.

Apart from these social factors, ecological factors play an important role in explaining a large amount of variance in the sex ratios of many different species. In many fish and amphibia, both genetic and environmental factors are important in sex determination (Blazquez et al., 1999). In some reptiles, sex determination is nearly entirely dependent on temperature (Janzen & Paukstis, 1991), although genetic and environmental factors may interplay in some taxa (Solari, 1994). Sex determination and hence primary sex ratios in amphipod crustaceans can be dependent on photoperiod (Adams, Greenwood & Naylor, 1987), while the size of a host plant influences ratios in some herbivorous insects (Craig, Price & Itami, 1992). For trematodes, the number of conspecifics within a host plays a role in determining sex ratios (Trouvé et al., 1999), while in parasitoid wasps host size is important (Napoleon & King, 1999). Local and temporal variation in these environmental factors can produce large skews in sex ratios.

There are a number of anthropogenic causes for abnormal sex ratio skews in animal or plant populations. Global temperature change is expected to change the sex ratio in species with temperature dependent sex determination (Hogg & Williams, 1996; Patino et al., 1996; Binckley et al., 1998). Sex differences in behaviour and size can lead to sex ratio changes as a result of selective hunting or trapping (Roosenburg et al., 1997; Beets & Friedlander, 1999), an effect that often occurs accidentally but is sometimes used intentionally, for example as a method for pest control (Robinson, 1983). It may also be proposed in the course of a programme for disease control (Krebs et al., 1998). Even a conservation programme can lead to a dramatic change in sex

ratio for various reasons (Saltz & Rubenstein, 1995), or small and endangered populations are just found to be skewed in their sex ratio (Lens et al., 1998; Eldridge et al., 1999; Godfrey et al., 1999).

The potential for manipulation

There are a number of potential non-invasive ways to influence family sex ratio. Obviously, if sex determination is influenced by ecological factors, manipulating these factors means manipulating sex ratio. In turtles and other reptiles, for example, eggs could be collected and incubated at temperatures that result in the sex ratio one wishes to produce, or the temperature at egg laying sites could be artificially changed (Girondot, Fouillet & Pieau, 1998). Manipulation of sex ratio by changing incubation temperature is also possible in some amphibia (Solari, 1994).

The potential for non-invasive sex ratio manipulation is far less obvious in species in which sex chromosomes mainly determine the gender of an offspring, like in all birds and mammals. However, a number of findings in behavioural ecology suggest that non-invasive sex ratio manipulation could be possible by influencing the social environment of a female. For example, it should be possible to manipulate species where females adjust their family sex ratio in response to a perceived skew in the population sex ratio. In many controlled environments (e.g., in zoos) a skewed population ratio could be simulated by removing members of one sex and housing them separately (Perret, 1996). Alternatively, sensory stimuli that are used by the females to estimate the population sex ratio, for example the number of different types of urine they are exposed to, could be explored to simulate increases in one of the genders (Perret, 1996).

In species where male characteristics play a role in sex determination, it could potentially be useful to exploit the rules used by females to determine the attractiveness of a male. It is rather unlikely that the decision rule about whether a given male is perceived as attractive or not is entirely genetically fixed (Real, 1991). In most species such a decision rule may be adaptable to a certain degree to a sampling template given by the population, by the female's personal experience, or by costs imposed on female choice (Milinski & Bakker, 1992). If, for example, females have a general preference for males with a red belly, then a male with a medium red belly might be perceived as very attractive in a population of dull males, but as unattractive in a population of very red males. To make the female produce more sons to a medium red male in response to his attractiveness, it may be useful to avoid exposing her to really beautiful males before mating. Analogously, exposing her to some (real or dummy) very beautiful males before mating could potentially cause her to produce more daughters from a male that is now perceived as not very attractive. Moreover, instead of changing the template a female uses to judge the relative attractiveness of a male, it may sometimes be possible to alter the attractiveness of the male directly, for example by cutting or attach-

ing feather ornaments, or by changing the light conditions during mating (e.g., using green light to neutralize the red colour of many ornaments). However, it will often be necessary to test whether such an option for non-invasive manipulation exists in a given species.

A more invasive method, and therefore a probably less attractive one for conservation, would be to manipulate the females' condition in species where female condition is likely to influence family sex ratio. Condition could be manipulated by a changed feeding regime. If the manager wishes to get more females, however, intentionally decreasing female condition bears the danger of weakening her to a degree that she might become more vulnerable to diseases or other stress factors. This would need to be balanced against the potential benefits of the manipulation.

Hormone treatments have been successfully used to produce functional sex reversed embryos or larvae that are genetically females or males. This is possible in many fish (Solari, 1994; Horvath & Orban, 1995), amphibia (Solari, 1994) and reptiles (Solari, 1994; Blazquez et al., 1999). However, there are also disturbing or even dangerous physiological side effects possible as a consequence of hormone treatment (Girondot et al., 1998). Functional sex reversed females or males have been used successfully in heterogametic species to produce all homogametic offspring. A homogametic female that has been sex reversed by hormonal treatment to a functional male can only produce daughters when mated with a homogametic and functional female. This trick is often used in fish culture (Crim & Glebe, 1990).

Sometimes, saving an endangered species from extinction requires the use of assisted reproductive technology (ART) (Gibbons et al., 1995; Dobson & Lyles, 2000; Lanza et al., 2000). Although the techniques and protocols do not normally seem to influence sex ratio in humans (Gray et al., 1998) or pigs (Soede, Nissen & Kemp, 2000), some methods of micromanipulation or freezing and some in vitro culture conditions may have an effect on sex ratio at birth in mice and cattle (King et al., 1992; Gutierrez et al., 1995). These first observations need to be better understood. Eventually, sex ratios could be manipulated by adding another technical step to ART protocols already in use.

Methods for sexing sperm and separating sperm that would lead to a female or to a male embryo are available (review in Seidel, 1999), and these methods are being continually improved today (Blecher et al., 1999; Welch & Johnson, 1999). Even cryopreservation of sexed sperm is possible and already used in agriculture (Schenk et al., 1999). Other methods for sex ratio manipulation involving molecular techniques have been discussed by Wilmut et al. (1992).

TOWARDS AN OPTIMAL MANIPULATION

General aspects

The more a population decreases in number, the more likely it is that the mean fitness of its members, and

thereby the long-term survival expectancy of the population, decreases owing to genetic problems (Lande, 1998). These problems include (1) an increase in inbreeding and hence an increase in inbreeding depression, (2) a general loss of genetic variability and therefore a reduced potential for adaptation to changing environments, and (3) the risk of fixation of deleterious mutations owing to random drift. Therefore, an obvious aim in conservation is to stop this decrease in number of individuals or even to achieve population growth. Since females are normally the limiting source of offspring, population growth in terms of actual number of offspring would be increased with every additional offspring that is a female instead of a male, as long as there are enough males available to fertilize the eggs of all these females.

However, an important drawback of manipulating the sex ratio of a population away from equality is the fact that the genetic problems outlined above are only indirectly dependent on the population size, and instead are directly related to the genetically effective population size N_e (e.g., Hartl, 1987). This measure is therefore an important parameter in population genetics and conservation biology (Caughley & Gunn, 1996: 167–170). N_e is the size of an ideal population that loses genetic variance at the same rate as does the real population. Hence, N_e corrects for a number of factors, such as age-related differences in reproductive rates, unequal family size, and, of interest here, unequal numbers of males and females. These corrections are necessary, because any skew in sex ratio increases the variance in individual reproductive success. Such an increase in the reproductive variance leads to increased inbreeding and thereby to a loss of genetic variability in a population.

An empirical example of the negative effects of skewed sex ratio in conservation is the success or failure of ungulate reintroductions (Komers & Curman, 2000): the population survival of small, heavily female-biased populations was more variable than where sex ratios were more equal. Such female-biased populations also grew on average more slowly. This could be due to increased intra-sexual conflicts and/or increased inbreeding.

Optimizing population growth in terms N_e

In the following I will assume that the only and best measure of a successful population growth is the effective population number N_e (this assumption certainly reduces the complexity of the problem; see also the Discussion). By manipulating the sex ratio of the first generation ($t = 1$) away from equality we get a reduced N_e as compared to the unmanipulated situation, because

$$N_e = 4N_mN_f/(N_m + N_f) \qquad (1)$$

(e.g., Hartl, 1987). However, with continuing sex ratio manipulation, N_e at generation $t = 2$ or later can be above the N_e of an unmanipulated population, because the increase in the absolute number of individuals in a population can, at some sex ratios, outweigh the effect the skewed sex ratio has on N_e. This benefit of certain sex

ratio manipulations still exists when the genetic bottleneck that has been created by the manipulation for the first generation ($t = 1$) is taken into account in the calculation of N_e. This is done by combining the effective numbers of many generations with the formula

$$1/N_e = (1/t)(1/N_1 + 1/N_2 + \ldots + 1/N_t) \qquad (2)$$

(Hartl, 1987). If the right kind of continuous sex ratio manipulation is chosen, N_e can be higher for the manipulated population from the second or third generation after the start of the manipulation onwards (Fig. 1). Stopping the manipulation later has a beneficial influence on N_e if the sex ratio returns towards equality.

To get this increase in N_e as compared to a population with equal sex ratio requires a decrease of N_e in the first generation ($t = 1$) following the start of the manipulation, as compared to an unmanipulated population at equal sex ratio. In other words, any manipulation away from a 1:1 sex ratio in order to increase population growth sends the population through a genetic bottleneck for at least one generation. The only possible short-term benefit from this is that some recessive mutations may be purged, resulting in a decline of inbreeding depression (Kirkpatrick & Jarne, 2000). However, Byers & Waller (1999) showed that purging is an inconsistent force within populations, and Ballou (1997) concluded that purging has only small effects in captive populations.

A simulation study: methods and results

In the following, I first use a simple simulation study to examine the effect of different kinds of sex ratio manipulation, in populations with various growth rates, on the
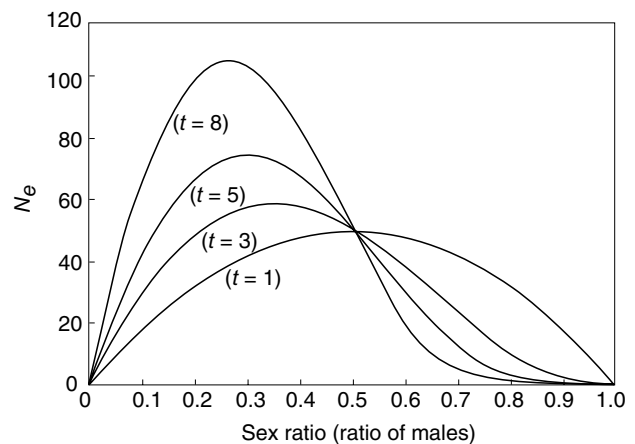

Fig. 1. The effect of different sex ratios on the effective population size N_e (see equation 2). The effect is shown here for a starting population of 25 males and 25 females at $t = 0$ and a constant lifetime reproductive rate of two offspring per female. Maximum N_e at generation $t = 1$ is only reached with a 1:1 sex ratio, but a continuous sex ratio manipulation towards a female bias leads to enhanced population growth in terms of absolute (or demographic) population numbers N_a. High N_a can eventually compensate for the initial genetic disadvantage of an unequal sex ratio. This can lead to increased N_e's, but only in further generations.

absolute (i.e., demographic) population size N_a, and on the genetically more relevant N_e on the long run. I then include the fact that the population size often interacts with the population growth or decline. This is especially so in small populations, where any decline is normally not linear but exponential ('Allee effect').

Take, for example, a starting population at $t = 0$ of 50 males and 50 females ($N_a = N_e = 100$). Each female has a lifetime reproductive rate of n offspring, and, for simplicity, there is no intra-sexual variance in reproductive success. Any sex ratio manipulation has an immediate effect on the sex ratio of the next generation at $t = 1$ and hence on N_e at $t = 1$ (see equation 1). N_a can only be influenced by the manipulation at $t = 2$. The manipulations can be stopped at any time, after which the sex ratio in the population switches back to 1:1.

Figure 2 shows the effect of some arbitrary selected types of sex ratio manipulations on N_a and on N_e. It becomes clear from the figure that a sex ratio manipulation is most effective in populations that would decline or at least not grow if left unmanipulated. Moreover, a manipulation during only two generations (Fig. 2(d)–(f)) can already produce strong effects and sometimes save

a declining population for some more generations (Fig. 2(e)). A continuous sex ratio manipulation over many generations (e.g., Fig. 2(g)–(i)) produces very high N_a's compared to the resulting N_e's, and hence may often not be very practical.

The following formula is used to include an Allee effect into the simulation:

$$n = c_1 + c_2 * \ln(\text{number of females in a population}) \quad (3)$$

where n is again the number of offspring per female, and c_1 and c_2 are constants. This formula assumes that the Allee effect depends primarily on the number of females in a population. The effect starts if the number of females in a population is below a critical number N_{crit}.

Figure 3 demonstrates that if an Allee effect is included, any sex ratio manipulation not only increases N_a and, in the long run, N_e, but also shifts the population quickly into population numbers that are relatively safe against the Allee effect (Fig. 3). Again, a manipulation during only a few generations can already produce strong effects.
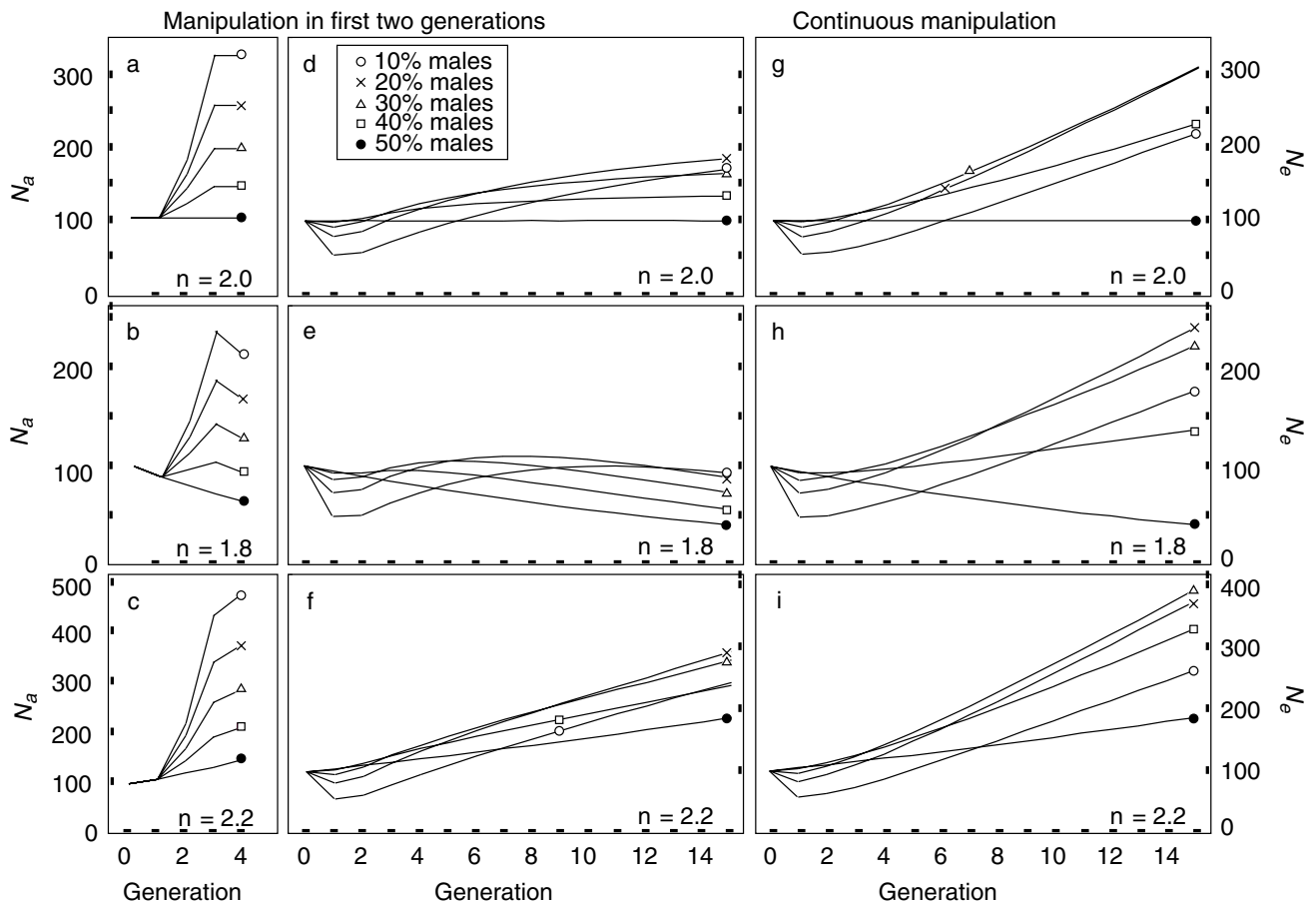

Fig. 2. The effect of different sex ratio manipulations on the absolute population size N_a ((a)–(c)) and the effective population size N_e ((d)–(i)) as calculated in equation 2, i.e. taking any initial genetic bottleneck into account. In (a)–(f), the offspring sex ratio is manipulated in the first two generations only, after which it switches back to 1:1 (i.e. equal sex ratios from generation 3 onwards). In (g)–(i), the manipulation is continuous. The figures also explore the effect of different population growth rates, i.e. the mean number (n) of offspring per female. No interaction between population size and growth rate is assumed here. The figures show the effect of an arbitrary selected sample of sex ratios as explained in the insert in (d).
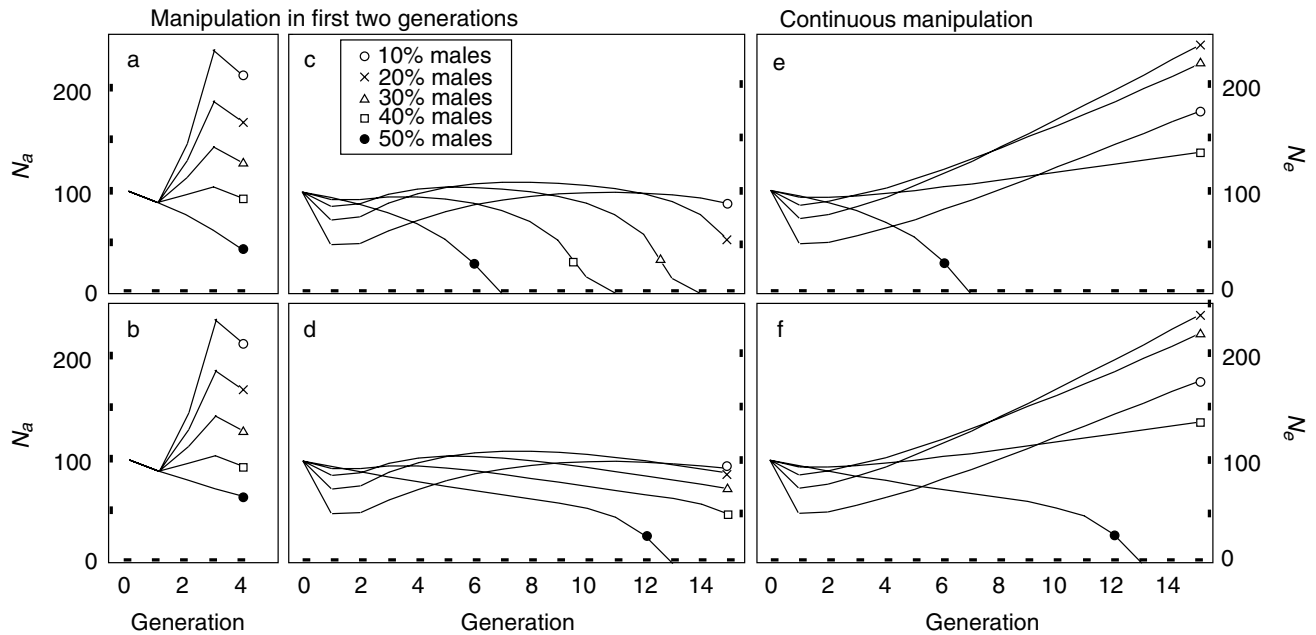
Fig. 3. The effect of different sex ratio manipulations in declining populations that suffer from the Allee effect, i.e. from an exponentially increasing population decline. Analogous to Fig. 2, (a)–(d) show the effect of a sex ratio manipulation during the first two generations only, while (e) and (f) show the effect of a continuous manipulation. The symbols are explained in the insert in (c). In all examples, each female gets $n = 1.8$ offspring when the number of females in a population is above a critical number N_{crit}. If it is below N_{crit}, the Allee effect starts (see equation 3). Parts (a), (c) and (e) describe a rather strong Allee effect with $N_{crit} = 50$, $c_1 = -1.5$, and $c_2 = 0.85$. In (b), (d) and (f), the Allee effect is weaker with $N_{crit} = 20$, $c_1 = -1.5$ and $c_2 = 1.0$.

DISCUSSION

There are a number of potential ways to manipulate the sex ratio of a population. As the sex ratio of a population is directly linked to its potential for growth, manipulating sex ratio away from too many sons towards at least a 1:1 ratio may be in general beneficial for an endangered population. A sex ratio skewed towards more females not only would increase population growth but could, especially in some large mammal species like rhinos, elephants or tigers, lead to a reduced conflict between animals and humans. It is often the males who produce most of these conflicts because they tend to be, especially in polygamous species, more risk-prone than the females (Sukumar, 1991).

However, there are also severe risks involved in manipulating sex ratios. First, maintaining only small numbers of males increases the chances that the whole male population goes extinct due to, for example, stochastic fluctuations, epidemics or accidents in housing. It is, in principle, also possible that some kinds of sex ratio manipulation affect processes of sexual selection (Clutton-Brock, Rose & Guinness, 1997; Jirotkul, 1999; Jiggins, Hurst & Majerus, 2000). This bears the risk of losing culturally transmitted characteristics of what would be the natural breeding system. It could even create genetic selection against the natural breeding systems. Further possible consequences are a change of competition in sex-specific use of resources (Gwynne, Bailey & Annells, 1998), or a general change of sexual genetic evolution (Owens & Thompson, 1994;

Girondot *et al.*, 1998) since the evolution of primary sex ratio is expected to be under frequency-dependent selection (Charnov, 1982; Conover & Van Voorhees, 1990).

By changing the sex ratio towards increased numbers of daughters, a population will experience a decrease in N_e of at least one generation. This kind of sending a population through a temporarily increased loss of genetic variance may be a rather drastic measure. In the long run, however, such a manipulation may result in an increase in N_e and hence in reduced further loss of genetic variance as compared to the unmanipulated situation.

The potential future benefits and current risks of a sex ratio manipulation need to be carefully optimized. Such an optimization is not a trivial problem as it depends on a number of parameters that are likely to differ from species to species (e.g. the potential for manipulation, population growth rates, carrying capacities, etc.). It will therefore be necessary to find an optimal sex ratio manipulation separately for each case. It may nevertheless pay to re-evaluate some methods of supportive breeding in the light of the possibility of increased population numbers (and decreased inbreeding) over a longer time span. This may result in a more effective breeding programme for endangered species and may help to keep at least 90% of the founders' heterozygosity over a period of 200 years (Soulé *et al.*, 1986). In cases where the sex ratio of a small population was already skewed prior to intervention, any manipulation of the sex ratio of future generations may even be beneficial from the beginning.

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