Update TRENDS in Ecology and Evolution Vol.22 No.9

Research Focus

Control of introduced species using Trojan sex chromosomes

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To control introduced exotic species that have predominantly genetic, but environmentally reversible, sex determination (e.g. many species of fish), Gutierrez and Teem recently modeled the use of carriers of Trojan Y chromosomes — individuals who are phenotypically sex reversed from their genotype. Repeated introduction of YY females into wild populations should produce extreme male-biased sex ratios and eventual elimination of XX females, thus leading to population extinction. Analogous dynamics are expected in systems in which sex determination is influenced by one or a few major genes on autosomes.

Exotic species and biological control

Exotic invasions often occur after the accidental or planned release of non-native species (e.g. Box 1), and are believed to be second only to habitat destruction as a threat to biodiversity [1]. Methods of dealing with exotics are diverse and each can generate additional problems. For example, traditional biological control — the introduction of secondary controlling species — often causes undesirable non-target species effects and indirect ecological damage [2]. Similarly, the use of selfish genetic elements to increase mutation loads or bias sex ratios in the exotics requires care to avoid evolved resistance or their horizontal transfer to other species [3]. The introduction of inducible fatality genes — neutral genes that become lethal when activated by an external agent or stimulus — is also problematic, as they are required to become fixed or achieve high frequencies in the population before activation [3]. In addition, transgenic organisms are typically viewed with suspicion by the general public. The sterile male release technique — whereby sterile males are released *en masse* and out-compete wild males for matings with females — is generally limited to insects and can suffer from the problem of reduced attractiveness of sterile males [4]. Recently, however, a novel method ('Trojan' sex chromosomes; [5]) has been proposed for use in exotic fish populations by Gutierrez and Teem [5] that is potentially free from these drawbacks.

Improved methods of control

One of the unfortunate properties of many current controlling techniques is that, once released, the introduced controlling agent, be it a species or a genetic construct, is free to spread unchecked in the host population or ecosystem. These methods can therefore be considered irreversible and, hence, should be initiated only after careful consideration of their potential effects. Moreover, to restore ecological equilibria, or for practical or economic reasons, some problem populations require a managed reduction in size rather than complete eradication [1]. Thus, the ability to adjust the degree of control, either up if its progress is less than expected or down if it is having unwanted side effects, is desirable. This suggests the need for a method of control that: is intrinsic to the problem population (i.e. has no non-target or indirect ecological effects); can be tailored dynamically to reflect the current or desired circumstance; and has a recoverable quality (so that the effects of control can be reversed if required).

Trojan sex chromosomes

Recently, Gutierrez and Teem [5] modeled the repeated introduction of individuals that are phenotypically sex reversed from that expected given their genotype (i.e. carriers of so-called Trojan sex chromosomes; [5]). They showed it to cause a disproportionate influx of one sex chromosome into subsequent generations, biasing the sex ratio and leading to potential population extinction. In some fish species displaying male heterogamety (where XY = males and XX = females), viable females carrying two Y chromosomes can be created over two generations using estrogen treatments during early development (Box 2). These YY females can then be released into an exotic population to mate with normal XY males. The resulting progeny comprise only XY and YY males, and the population sex ratio becomes male biased. This deviation from sexual unity increases in subsequent generations owing to the presence of the YY males, whose offspring will be all XY males (when mated with an XX female) or all YY males (when mated with an introduced YY female). Further introduction of even small numbers of YY females into the population might therefore be sufficient to cause an ever-increasing sex ratio bias towards males and a diminishing number of true (XX) females. The efficacy of eradication depends on the life history of the target species, but Gutierrez and Teem [5] estimate that an input of YY females of $\sim$3% of the population would be sufficient to cause extinction in a population of several hundred individuals within a few decades. The speed of extinction of the population increases with the number of YY females introduced and with heightened rarity of XX females (e.g. as a result of chance loss or because extreme male-biased sex ratios increase deleterious harassment of females by males

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Available online 20 July 2007.

Box 1. The increasing threat of invasion from aquaculture and fisheries

Aquaculture, the farming of marine or freshwater species for human consumption or use, is expanding rapidly and increasing with it is the risk of exotic invasion from escapees [14,15]. In addition, lakes and rivers are frequently stocked, often with salmonids such as rainbow trout *Onchorynchus mykiss*, brown trout *Salmo trutta* and brook trout *Salvenius frontinalis*, for recreational fisheries. Such introductions can have deleterious effects on endemic faunal biodiversity, with amphibian species being particularly prone [16,17]. As a result of their undesirable effects, many countries have tried to impose strict controls on non-native aquatic species (e.g. in the UK [http://www.defra.gov.uk/fish/freshwater/pdf/species.pdf] and Switzerland [*Verordnung zum Bundesgesetz über die Fischerei, Anhang 3*; http://www.admin.ch]). Promisingly, however, the removal or loss of introduced species can often result in recovery of the native faunas [16,18]. A mechanism of bio-control, such as that provided by Trojan sex chromosomes, is therefore needed to counteract the influence of these ecological exotics.

[6]). However, the sex ratio returns to unity if the influx of YY females ceases before elimination of XX females [5].

Trojan sex chromosome theory [5] was initially developed to tackle the increasing problem of exotic fish species (Box 1). However, the requisite phenotypic and chromosomal engineering of sex potentially exists in a variety of taxa with predominantly genetic, but environmentally reversible, sex determination, including crustaceans [7], amphibians [8] and, theoretically, reptiles [9], making its applicability potentially taxonomically wide ranging. Nonetheless, advances in the manipulation of sex in these taxa lag behind that seen currently in fish. Although the original model [5] envisaged genetic sex determination via distinct sex chromosomes, we believe that similar dynamics will also be observed in systems in which sex is dictated by one or a few major genes.

Box 2. Creation of fish containing Trojan sex chromosomes

Many fish species show genetic sex determination via sex chromosomes or major sex-determining genes whose effects can be reversed by extreme changes in the environment. In aquaculture, it is common practice to manipulate sexual phenotypes by exposure to sex hormones because the sexes often differ in their growth rate and it is preferable to culture one sex or the other to maximize yield; genetic (XY) males can be feminized with estrogens to become phenotypic females and genetic (XX) females can be masculinized to phenotypic males by exposure to androgens [10,11].

The most complete example of Trojan YY female production is that of Nile tilapia *Oreochromis niloticus* [12]. To create females containing Trojan sex chromosomes (Figure I), genetic (XY) males are first feminized through the application of estrogens and the resulting XY females are then crossed with standard XY males. One-quarter of the subsequent progeny comprises YY males. These individuals can be distinguished from XY and XX offspring either by genetic screening (e.g. using X- and Y-linked genetic markers) or by the sex ratio of their progeny when mated to normal XX females (YY individuals produce only sons). To generate YY females, YY males are first mated to feminized XY individuals, and then the resulting progeny are treated with estrogens to produce equal numbers of YY and XY females [12]. Again, YY individuals can be identified by genetic markers or progeny sex ratio. Alternatively, YY males and females can be isolated by treating half of the progeny of the first XY female–XY male cross with estrogen; on average, 12.5% of the offspring will be YY males and 12.5% will be YY females. Again, YY genotypes can be identified either by using genetic markers or by scoring the sex ratios of their progeny when mated with wild-type individuals (YY individuals do not produce daughters). Once YY males and females have been generated, a stock population of YY individuals can be maintained by feminizing half of the YY offspring produced each generation [12]. A proportion of the YY females created by these processes can then be released into a wild population to deliver the Trojan sex chromosomes.

The strength of Trojan sex chromosomes

The advantages of the Trojan sex chromosome method are clear. First, the raw materials (i.e. YY females) can often be produced through adapted hatchery practices (Box 2). The method is intrinsic to the target species, so there is no probability of collateral ecological damage [2]. The genetic manipulations comprise a simple re-assortment of pre-existing sex chromosomes among individuals and so pose little external threat. Fewer individuals need to be released than in the sterile male technique, as its success relies not on competition over matings but on multiple genetic effects that extend beyond the life of the individual

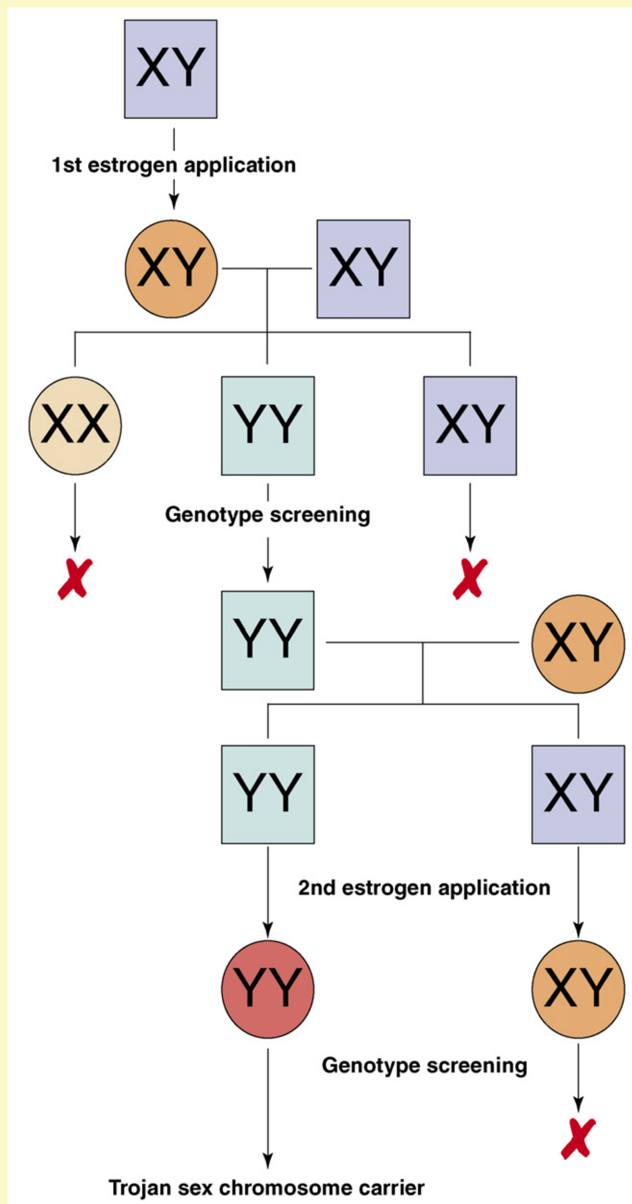

Figure I. Schematic outlining a method of YY female production.

and spread, albeit transiently, in the population. And because the reduction in population size is linked to the magnitude and frequency of YY female introduction, control can be tailored from mild management to complete eradication. The consequences of YY female additions are also non-permanent (excepting XX extinction, of course), so unexpected or unwanted effects of management can be reversed by cessation of YY input.

The Achilles' heel of Trojan sex chromosomes?

The advantages described above make Trojan sex chromosomes attractive for those systems that enable the relevant manipulations of sexual phenotype. However, the model makes several assumptions, which require careful examination. Perhaps most importantly, YY and hormone-treated animals are assumed to have the same viability as wild-type individuals. If they have lower viability, then the method will not be as effective. Unfortunately, quantitative tests of viability differences between YY or hormone-treated and normal individuals are rare and somewhat inconclusive. For example, there are some reported increases in growth [10], but, equally, estrogen treatment has been shown to reduce adult growth, lower survival and impact negatively on fecundity in XY females [10,11]. Little is known about the relative viability of YY females. Reduced viability and survival is characteristic of YY males in some species, although not all (e.g. [12]). Similarly, the efficacy of the method will be compromised if there is any (evolution of) mating avoidance of YY or sex-reversed individuals by wild types. The method also relies on permanence and predictability of sexual phenotypes after manipulation. This might be problematic if sex determination in target species is influenced by many genes or if there are late-acting environmental effects on sex. Finally, if hybridization occurs between the exotic species and closely related native relatives, a reportedly frequent phenomenon [13], then the deleterious effects of Trojan sex chromosomes might spread beyond the target species. However, such effects are likely to be limited and can be alleviated by cessation of YY female introduction.

Conclusions

The potential setbacks of the Trojan sex chromosome theory merely highlight how little is known about the relative performance of hormonally or genetically manipulated individuals in the wild, or indeed about the sex-determining systems of many invasive species. As such, these uncertainties should act as a call-to-arms for more research on this promising mechanism to restore endemic biodiversity. In addition, the population dynamics of Trojan sex chromosomes can be evaluated under controlled conditions in real biological systems using multigenerational experiments on populations reared in the laboratory. Finally, as many of the exotic species are intensively studied as a result of their commercial importance, there are frequent opportunities to examine some of the questions raised here.

Acknowledgements

This article benefited from input from B Schmid, C Küng, P Friedli and three anonymous referees. Financial support was provided by the Swiss National Science Foundation.

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doi:10.1016/j.tree.2007.06.010