Obtaining snapshots of genetic variation using hemiclonal analysis

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Abstract

Hemiclones are naturally occurring or artificially produced individuals that share a single specific genetic haplotype. Natural hemiclones are produced via hybridization between two closely related species, while hemiclonal analysis in *Drosophila* is carried out in the laboratory via crosses with artificially created "clone-generator" females with a specific genetic make-up. Hemiclonal analysis in *Drosophila* has been applied very successfully to date to obtain measures of standing genetic variation for numerous traits. Here we review the current hemiclonal literature and suggest future directions for hemiclonal research, including its application in molecular and genomic studies, and the adaptation of natural hemiclonal systems to carry out *Drosophila*-type studies of standing genetic variation.

What is hemiclonal analysis?

Hemiclonal individuals are genetically identical for half of the diploid genome, and occur naturally in certain hybrid systems which are outlined in the next section. A hemiclonal laboratory system has also been developed in *Drosophila melanogaster*, and a hemiclonal analysis protocol for estimating quantitative genetic parameters has been formalized in this model organism [1]. Here our intention is to conduct a broad review of hemiclonal analysis as a quantitative genetic tool, regardless of taxonomic grouping, and to argue for increased capitalization on the advantages of hemiclonal analysis in both natural and artificial systems.

The production of a set of hemiclonal individuals can be thought of as analogous to fertilizing many eggs with the same genetically identical sperm (or alternatively, by letting many genetically identical eggs be fertilized by different sperm), producing individuals with the same haplotype expressed in a random genetic background (Figure 1). If multiple hemiclonal lines are captured from the same source population then it is possible to carry out screens of standing genetic variation, in essence capturing a "snapshot" of the available genetic variation for a given trait. Heritabilities, coefficients of additive genetic variation, and other quantitative genetic parameters can be calculated from hemiclone data using a design which partitions variance into within-hemiclone and between-hemiclone components [1]. Such hemiclonal heritability values will be approximately equal to one half the heritability in a normal diploid organism [1]. Because the hemiclonal haplotype is always inherited intact (i.e. unrecombined), it is not possible to separate additive genetic effects from certain types of epistatic effects within the hemiclonal genome, and the estimates of quantitative genetic parameters obtained from hemiclonal analysis must be considered as representing an upper bound with respect to the additive genetic variance (see [1] for details). Since this portion of the epistatic variance should be small relative to the additive genetic variance, this is unlikely to be a major issue.

Despite this potential disadvantage, hemiclonal analysis also provides a number of unique advantages and should be seen as a useful complement to standard breeding designs for parameter estimation in quantitative genetics (e.g. North Carolina, diallel, etc.). For example, hemiclonal systems have the ability to produce an almost unlimited number of individuals with the same haplotype. This makes it possible to accurately measure even very low levels of genetic variance (e.g. [2]), and allows the splitting of hemiclones into multiple treatments without having to trade-off treatment number and sample size per treatment (e.g. [3]). Another major advantage is the ability to test the same known haplotype in different genetic "environments", for example in combination with different mitochondrial strains, in an inbred or outbred state (e.g. [4]), or by expressing them in males versus females to look at sex-specific effects (e.g. [1]). It is also possible to preserve specific hemiclonal lines for many generations so that follow-up experiments can be carried out on exactly the same set of haplotypes [2,5–7].

Natural hybrid hemiclone systems

The first hemiclonal hybrid (or hybridogenetic) system was discovered by Schultz [8], and the term "hemiclone" (which was coined by Klaus D. Kallman) was first formally applied to such a system by Vrijenhoek and colleagues [9]. There are several different groups of natural hemiclonal hybrids, and the most well-studied of which are Livebearing toothcarps *Poeciliopsis*, the edible frog *Pelophylax esculentus* (formerly known as *Rana esculenta* [10]), and *Bacillus* stick insects (Box 1). In these systems one parental species genotype is excluded from gamete production, and all eggs or sperm contain identical copies of the other parental genotype [11–14]. In all three groups it is the genome of the maternal parent in the original hybrid crosses (*Ps. monacha*, *Px. ridibundus* where *Px. esculentus* co-occurs with *Px. lessonae*, or *B. rossius*) that is maintained and passed on hemiclonally

[11,12,14–17], and these species can be considered the "gametogenic" parental species. By similar reasoning the species whose genome is excluded every generation can be considered the "somatic" parental species (*Ps. lucida/occidentalis/latidens, Px. lessonae*, or *B. grandii*), and these are the terms we will use here. Because it is exclusively the genome from the gametogenic parental species which is used in gamete production, the only way these hybrid species can be maintained is by mating with the somatic parental species every generation.

Hybridogenesis appears to be a spontaneous by-product of the genetic characteristics of the parental species genomes [11], and it is genetic factors which determine which parental species is the gametogenic and which the somatic species in *Poeciliopsis* and *Bacillus* hemiclones [12,13]. Although reciprocal hybrid crosses are possible in the *Pelophylax* system, *Px. ridibundus* is much larger than *Px. lessonae*, making *Px. ridibundus* much more likely to be the maternal parent in the initial hybridization [14]. Variation in the propensity to produce hemiclonal hybrids has been shown in this system [18–20]. The parental species which form hemiclonal hybrids are not usually in contact today, so the hybridization events which have produced hemiclonal hybrid species are usually dated as having occurred several thousand years ago. However new hemiclones can be produced where the parental species encounter one another via artificial introductions, or in narrow stable hybrid contact zones [11,15,21]. Synthetic hemiclonal hybrids can also be produced in the laboratory via *de novo* hybridization between parental species [11,18]. Much of the research on hemiclonal hybrids has naturally focussed on their unique properties, and for example includes investigations of mutation accumulation within hemiclones (e.g. [22–28]), interactions between hybrids and their parental species (e.g. [29–31]), conservation biology of hemiclonal hybrids (e.g. [15]), the mechanism(s) of exclusion of the somatic parental species genome [16,17], maternal provisioning strategies (e.g. [32]), or effects of backcrossing to the gametogenic parental species (e.g. [24]). These

sorts of studies have recently been reviewed elsewhere [12–15], so here we will mainly focus on studies of genetic and phenotypic variation among hemiclones.

Coexistence of hemiclonal hybrid species and their somatic parental species suggests that the two should be phenotypically distinct from each other, and this is indeed often the case [14,33,34]. Multiple hemiclones can also coexist within the same population [11,14,35], and two hypotheses have been formulated to explain the persistence of specific hemiclones through time: the Frozen Niche Variation (FNV) hypothesis and the General-Purpose Genotypes (GPG) hypothesis. The FNV hypothesis suggests that different hemiclones are adapted to different environmental conditions and can coexist via specialization and niche partitioning [36]. The GPG hypothesis, in contrast, suggests that successful hemiclones (i.e. those that persist through evolutionary time) are generalist genotypes that are adapted to a wide range of conditions [37]. These hypotheses are not mutually exclusive, however, and evidence supporting both processes has been found in natural populations [37,38]. A number of studies have investigated phenotypic differences between hemiclones (often in the context of the FVN and GPG hypotheses), and these studies can give us some insight into the standing genetic variation for these traits in the gametogenic parental species. Traits which have been compared and found to differ between *Poeciliopsis* hemiclones include female attractiveness (to males of the somatic parental species) [39], genital pigmentation, predatory efficiency, food preference, sexual aggressiveness [11], survival, fertility [40], length at birth, weight at birth, juvenile growth rate, brood size [7], genital morphology [6], thermal tolerance [41], juvenile avoidance behaviour (of cannibalistic parental forms) [42], and reproductive mode (matrotrophy or lecithotrophy) [43]. *Pelophylox* hemiclones have been found to differ in habitat preference and niche breadth [38], food consumption [44], survival to metamorphosis [24,45], growth rate, developmental rate [24], body mass at metamorphosis [45,46], time to metamorphosis [45], hind leg length, and jumping performance [46]. Time to metamorphosis and jumping performance also exhibited

genotype by environment interaction [45,46]. Overwinter survival did not differ between *Pelophylax* hemiclones in one study, although this might be an artefact due to low statistical power [34]. No phenotypic comparisons of *Bacillus* hemiclones appear to have been carried out.

The *Drosophila melanogaster* hemiclone system

In the 1990's, William Rice developed an artificial hemiclone system in *Drosophila melanogaster* which mimics the properties of natural hemiclonal systems [47]. It is similar to natural systems in that a single haploid genome is transmitted clonally, but instead of relying on hybridization this system takes advantage of some unusual chromosomal constructs that are available within *D. melanogaster*. So-called "clone-generator" females possessing two linked X-chromosomes and marked, translocated autosomes are the essential feature of the system. In short, clone-generator (CG) females are first crossed to wildtype males. The male offspring of this cross will have one wildtype haploid genome and one CG genome. A single F1 male is then crossed to several new CG females. This results in amplification of the wildtype genome (in terms of the number of individuals carrying it) which was captured in the first cross. The amplified hemiclonal genome can then be expressed as either sex in a random genetic background for analysis (Box 2).

Apart from the general advantages relative to standard breeding designs (which are commonly used in all the taxonomic groups discussed here) listed in the introduction, hemiclonal analysis also has some advantages over other methods that are more specific to *Drosophila*, such as the use of inbred lines, balancers, or introgression of specific chromosomal variants. Inbred lines are time-consuming to produce and can represent a skewed subset of the extant variation due to genetic purging during the inbreeding process. In contrast, hemiclonal analysis can be carried out in a short time and represents a truly random selection of wildtype variation within the source population, expressed in

a fully heterozygous state. This variation covers all major chromosomes, in contrast to introgression techniques which typically only focus on one chromosome at a time (e.g. [48]). Unwanted recombination, which can be a problem when using balancers [49], is also completely eliminated during hemiclone production because males are used to pass on the hemiclonal haplotypes (males naturally do not exhibit recombination in *D. melanogaster*).

Because the hemiclonal genome is passed on from father to son and never expressed in females during amplification, male-limited evolution is also possible [50]. The method is essentially the same as for screens of standing genetic variation, except that the amplification stage is extended for many generations, and during this period selection among hemiclones occurs for genotypes that are relatively more fit when expressed in males. A small degree of recombination is also added in order to prevent hitchhiking of deleterious alleles and allow beneficial alleles from different hemiclones to combine. This is achieved by producing females with two different hemiclonal genomes, which then generate sons with a recombined genotype that are returned to the male-limited population [50].

What have we learned from hemiclonal analysis in *Drosophila*?

Since the *D. melanogaster* hemiclone system was first developed approximately 15 years ago, 21 studies using the technique have been published, the majority from 2005 onwards. A summary of these studies and their findings are presented in Electronic Supplement Table 1. Some interesting patterns are evident. For one thing, the majority of the studies have been carried out in the context of sexual selection and sexual conflict [1–5,47,50–61], and only a handful of studies have been carried out in other contexts [4,62–64]. It is from the first group that some of the most convincing evidence has been obtained that intralocus sexual conflict and sexually antagonistic genetic variation can play an important role in evolution. Screens of standing genetic variation have shown that the

158 fitness of a genome is often dependent on whether it is expressed in males or females
159 [1,5,53,57,59,60], and male-limited experimental evolution has demonstrated that the evolution of
160 sexual dimorphism is consequently constrained in many traits [47,50,51,58,61]. Evidence of
161 interlocus sexual conflict comes from studies which have shown that some traits which increase male
162 fitness (e.g. sperm offense) also decrease the fitness of their mates (e.g. female longevity) [2,54–56].
163 Although it is possible that sexual conflict is exacerbated in a laboratory situation [65], these studies
164 have still been groundbreaking in helping us understand sexual conflict.

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166 The few studies that do not fit in the context of sexual conflict or sexual selection are conceptually
167 diverse. One deals with costs of immunity [62], one with condition-dependence [63], one with
168 mutation accumulation and the power of recombination [64], and one with inbreeding depression
169 [4]. The very different contexts of these studies give a hint of the investigative potential of
170 hemiclonal analysis. Both these and a number of other studies have also split hemiclones into
171 different treatments: with or without recombination [64], inbred or outbred [4], with short- or long-
172 term exposure to males [54–56], in high or low larval density [3,63], or between limited and
173 unlimited resource (yeast) treatments [62]. There was significant genotype-by-environment variation
174 in almost all cases, consistent with the expectation that genotype-by-environment interactions
175 should be widespread [66].

176

177 As with studies of natural hemiclones, *Drosophila* hemiclone studies also suggest that most traits
178 exhibit significant genetic variation [1–5,52–57,59,60,62]. It is also worth noting that several studies
179 managed to demonstrate genetic variation specifically for fitness [4,5,53,59,60], and in some cases
180 there was evidence of sex-specific genetic architecture for fitness. Heritability levels for fitness-
181 related traits were low in most cases [1,2,54,56,62] (with some exceptions [59,60]), which is

consistent with the fact that it is generally difficult to detect additive genetic variation for fitness in natural populations [67,68].

Filling in the gaps

As can be seen from the above summary, there is a severe lack of hemiclone studies in *Drosophila* dealing with topics other than sexual conflict and sexual selection. This bias toward sexual conflict and sexual selection studies is a consequence of the fact that relatively few researchers have used the *Drosophila* hemiclone system to date, most of whom have sexual conflict and sexual selection as a major focus in their research. Hemiclonal analysis as such seems to be well-known to researchers in ecology and evolutionary biology since the 20 published studies have collectively been cited over 1100 times (mean citations per publication per year: 8.83, SD = 6.0). The clone generator stocks required to produce hemiclones are also freely available upon request from several different labs, so there is no reason why hemiclonal analysis should not become more widely used in future. In light of this, we would like to suggest some areas for future research where we think the use of *Drosophila* hemiclonal analysis could be particularly beneficial.

One obvious avenue of further research is in quantitative genetics. Because hemiclonal analysis allows very accurate measurement of quantitative genetics parameters, it can be used to measure levels of standing genetic variation and estimate heritabilities and genetic correlations for all types of traits. Some particularly interesting possibilities involve the genetic variance-covariance (**G**) matrix. For example, how well does the phenotypic variance-covariance matrix (**P**) estimate the **G** matrix? Although some studies have addressed this question [69] it is far from resolved, and using hemiclones to estimate the **G** matrix should increase power substantially. It was also recently proposed that within-sex **G** matrices should be more stable than the between-sex genetic variance-

covariance matrix (or **B** matrix) [70]. This could easily be tested by using hemiclonal analysis to compare the **G** and **B** matrices for different source populations of *D. melanogaster*. Another interesting possibility is using hemiclonal analysis to detect evolutionary lines of least resistance in *Drosophila* [71,72], and then testing whether they are a constraint using experimental evolution.

As we mentioned above, several hemiclone studies have exposed individuals from the same hemiclone to different environmental conditions. However all current studies have only used two environmental treatments. Another obvious avenue of further research using hemiclonal analysis is therefore in the study of reaction norms and plasticity. By exposing the same hemiclone to a range of environmental treatments it will be possible to accurately measure reaction norms and plasticity [73] of individual genotypes. How sex-specific differences in body size plasticity contribute to sexual size dimorphism is poorly understood in insects [74], so this is an ideal problem for investigation using hemiclonal analysis. Similarly, there is evidence that heritabilities can vary across environments (e.g. [75]), and hemiclonal analysis would make it possible to test what sort of environmental variation has the most influence on heritability levels. A related area is the investigation of life history trade-offs, which are often difficult to measure [76] and can be environment-dependent (e.g. [62]). By splitting hemiclones into various treatments and manipulating the investment in different life history traits, it should be possible to characterize trade-offs with a greater degree of accuracy than is possible using other methods.

Finally, hemiclonal analysis can be used in combination with molecular and genomic methods. When collecting genomic data on expression patterns, a potential problem is that spurious differences between groups can be introduced due to uncontrolled factors or stochastic effects, such as differences in developmental environment, environmental differences immediately prior to sampling, or differences in treatment or timing when harvesting mRNA [66]. This problem can be

reduced by using hemiclones because any suspected confounding effects (such as timing of harvest) can be controlled for by exposing members of the same hemiclone to different levels of the effect. The same is true of inbred lines, but studies of variation in expression patterns using inbred lines (e.g. [77]) suffer from the drawback that expression levels can be influenced by genome-wide homozygosity. In contrast, hemiclones have all the advantages of inbred lines but make investigations of standing heterozygous genetic variation in expression patterns possible (e.g. [60]). The same argument can also be made for QTL studies using inbred lines (e.g. [78]), where rare mutations with large phenotypic effects when homozygous might be overrepresented [79]. Hemiclones are clearly highly useful for studying genetic variation as it exists in natural populations using molecular and genomic methods.

Future directions with other hemiclonal systems

Hemiclonal hybrids can be seen as capturing and freezing standing genetic variation from the gametogenic parental species. The similarity between this process and the creation of hemiclones for analysis of standing genetic variation in *Drosophila* should by now hopefully be apparent. Problems with using naturally-occurring hemiclones to this end are that they will have accumulated mutations over time [22,23,26,27] (although this effect can be partially ameliorated by occasional recombination between hemiclonal genomes [80]), and that selection has been operating on natural hemiclones since the original hybridization occurred, making them poor estimators of current standing genetic variation in the parental species. However, since synthetic hemiclones can be produced in the laboratory, studies of standing genetic variation analogous to those from *Drosophila* should be possible. Researchers using hybrid hemiclones have occasionally suggested using synthetic hybrid hemiclones to study mutation load in the gametogenic parental species (e.g. [14,21]), but only a single published study has used this method to explicitly make inferences about genetic variation in the parental species [7]. The study was carried out in *Poeciliopsis*, and showed

256 that 10-50% of the phenotypic variation in several traits (length at birth, weight at birth, growth rate,
257 and brood size) could be attributed to genetic variation in the *Ps. monacha* genome. However
258 because these synthetic hemiclones were always expressed in a hybrid state (i.e. they were never
259 backcrossed to *Ps. monacha*) it was not possible to calculate quantitative genetic parameters from
260 this data. Note that although this and other studies of genetic variation in *Poeciliopsis* have used
261 inbred males, this is not strictly necessary since hemiclonal females can be crossed to outbred *Ps.*
262 *monacha* males, producing individuals with one hemiclonal genome and one random outbred
263 genome.

264

265 Although studies of standing genetic variation in the gametogenic parental species are potentially
266 feasible using any of the three natural hybrid hemiclonal systems we discussed above, each has its
267 own pros and cons. The *Bacillus* system is likely the least suitable as it can occasionally undergo
268 spontaneous androgenesis, resulting in the elimination of the clonal genome [13]. The *Poeciliopsis*
269 system has the advantage of a short generation time (ca. 3 months to sexual maturity), but can
270 require additional crosses to compensate for yolk-size differences between the parental species
271 [7,40]. Although the *Pelophylax esculentus* system has the disadvantage of having the longest
272 generation time (ca. 2 years to sexual maturity), it has other properties that make it promising in this
273 context. Firstly, *Px. esculentus* individuals are not unisexual and have an XY genetic determination
274 system, which provides some control over the sex of hemiclonal individuals (see Box 2). Secondly,
275 *Px. esculentus* (and to a lesser extent its parental species *Px. ridibundus*) is a common research
276 organism in studies of physiology, which means that detailed physiological information is available
277 for this system. In both the *Pelophylax* and *Poeciliopsis* systems crosses can also be carried out via
278 artificial fertilization (e.g. [24]), making it possible to bypass behavioural effects such as mate
279 preference.

280

Via hemiclone-based studies of standing genetic variation, in principle just about any sort of trait could be investigated, and as with *Drosophila*, reaction norms and life history trade-offs could be interesting areas for such studies. The *Poeciliopsis* system is probably well-suited for this sort of study (e.g. [7]). The *Poeciliopsis* system could also be used to carry out female-limited evolution experiments, analogous to the male-limited evolution experiments that have been performed in *Drosophila*. One particularly exciting possibility is investigating interactions between nuclear and mitochondrial genes using the *Pelophylax* system. Recent work in *Drosophila* has shown that different mitochondrial genotypes can have profound effects on the fitness and expression pattern of identical sets of nuclear genes [81,82]. By crossing individuals from different *Pelophylax* hemiclones, it should be possible to produce individuals with the same nuclear genotype, but different mitochondrial genotypes (Figure 2; natural hemiclones may in fact occur in combination with mitochondrial DNA from either parental species [83]). Another possibility is using hemiclones to produce many individuals with specific phenotypic characteristics, for example for use in mate-choice studies. This would be similar to studies in *Drosophila* which have screened a number of genotypes to identify a subset with particular characteristics (e.g. high, low, or average male fitness, [2]) which were then used as a treatment in an experimental design.

Conclusion

Although hemiclonal systems have produced a number of very interesting results to date, there is definitely ample room for further research. Results from both natural and artificial systems suggest that there is significant standing genetic variation for a wide variety of traits, and hemiclonal analysis is particularly well-suited to determining the contribution of specific traits to fitness and detecting even low levels of genetic variance. Our aim with this review was to highlight the usefulness of hemiclonal analysis in obtaining snapshots of standing genetic variation, and we hope that both natural and artificial hemiclone systems will become more commonly used in this respect in future.

306

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Box 1: Summary of natural hemiclonal systems.

The Headwater livebearer *Poeciliopsis monacha* can hybridize with three different species to produce hemiclonal hybrids: the Clearfin livebearer *Ps. lucida* (hybrid *Ps. monacha-lucida*), the Gila topminnow *Ps. occidentalis* (hybrid *Ps. monacha-occidentalis*), and the Lowland livebearer *Ps. latidens* (hybrid *Ps. monacha-latidens*; Figure IA). These *Poeciliopsis* hybrids are always female, and so these species are unisexual.

The edible frog *Pelophylax esculentus* is a hybrid between the marsh frog *Px. ridibundus* (formerly *R. ridibunda* [10]) and the pool frog *Px. lessonae* (formerly *R. lessonae* [10], Figure IB), and has both male and female members. Similar systems are also found in *Px. hispanicus* (a hybrid between *Px. ridibundus* and *Px. bergeri*) and *Px. grafi* (a hybrid between either *Px. perezi* and *Px. ridibundus* or *Px. perezi* and *Px. esculentus*), although neither are as well-studied as the *Px. esculentus* system [15].

There are two hybridogenetic *Bacillus* species: *B. rossius-grandii grandii*, and *B. rossius-grandii benazzii*. Both are the result of hybridization between the Mediterranean Stick Insect *B. rossius* and *B. grandii* (Figure IC), but are produced from two different subspecies of *B. grandii* [84]. *B. rossius-grandii* hemiclones are effectively unisexual as well, because even though males can be produced they are always sterile [13].

These three systems are also members of species complexes which include polyploid parthenogenic (gynogenetic) members [12–14], and although there are additional hybridogenetic groups (i.e. Iberian minnows *Squalius alburnoides*, spined loaches *Cobitis*, oriental weather loaches *Misgurnus*,

and polyploid populations of *Px. esculentus* [12,19,85]), these species do not produce hemiclonal offspring, and will therefore not be considered here.

After an initial mating between a female from the "gametogenic" parental species (genotype GG) and a male from the "somatic" parental species (genotype SS; see text), a hybrid offspring with one haploid genome from each parent is produced (genotype GS; Figure ID). In hemiclonal species this hybrid offspring only produces gametes containing the gametogenic species genome (exclusion of the somatic species genome during gamete production is indicated by the broken line). In order to maintain the diploid hybrid genotype this individual must mate with males of the somatic species. The haploid gametogenic species genome does not undergo recombination, and is therefore clonally transmitted.

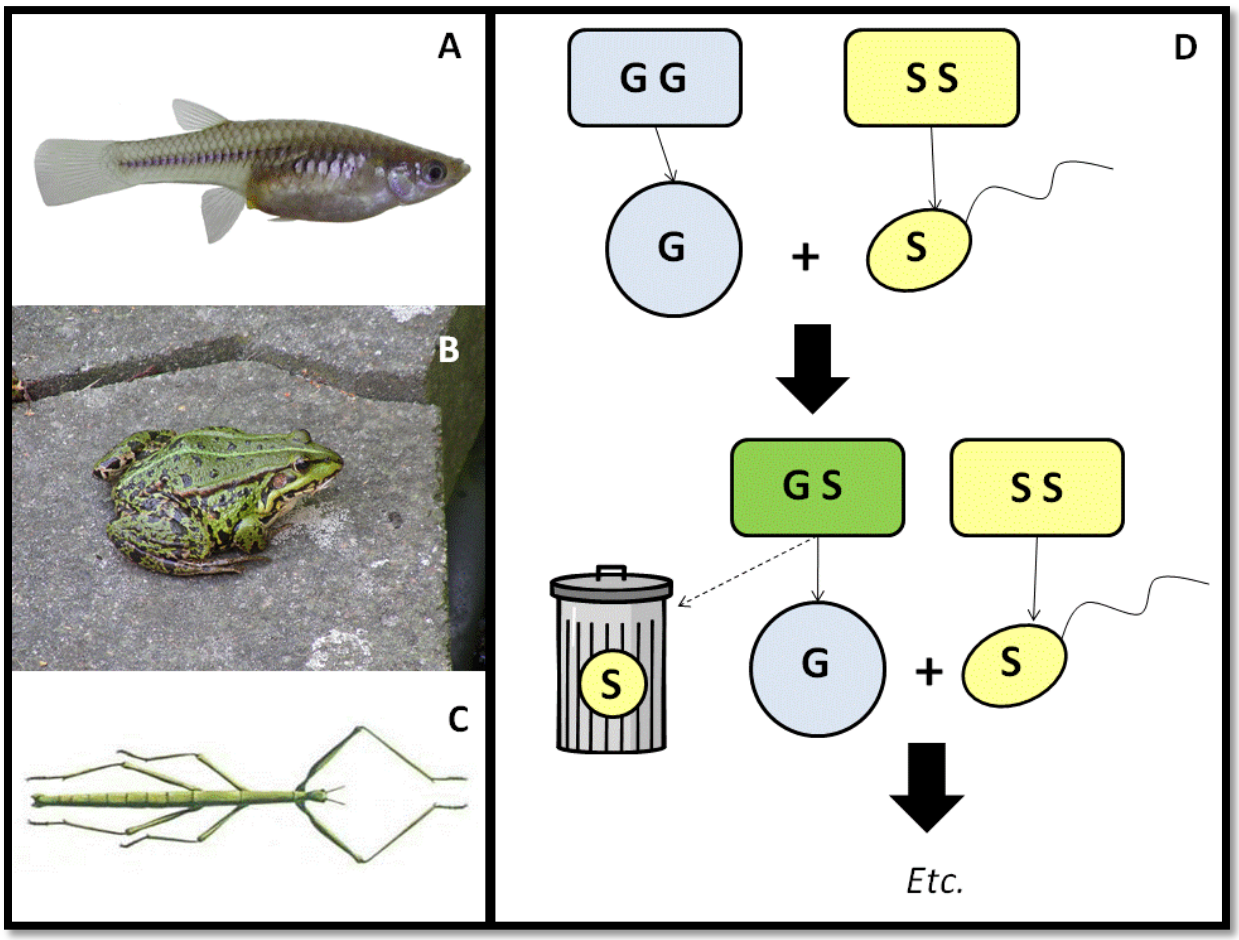


Figure I: Natural hemiclonal systems. A. *Poeciliopsis monacha-lucida.* Photograph kindly provided by Robert Vrijenhoek, Monterey Bay Aquarium Research Institute. B. *Pelophylax esculentus*. Photograph by Ib Rasmussen, obtained from Wikimedia commons (http://commons.wikimedia.org/wiki/Main_Page). C. *Bacillus rossius.* Picture obtained from Wikimedia commons (http://commons.wikimedia.org/wiki/Main_Page). D. Summary of the production and maintenance of hemiclonal species. A hemiclonal hybrid is produced by a mating between two parental species (genotypes GG and SS), and although this individual has received half its genome from each parental species (genotype GS) it only passes on genetic material from one of them to its offspring (egg with genotype G).

Box 2: Detailed description of hemiclone production in *Drosophila*

The production of hemiclonal individuals in *Drosophila melanogaster* is made possible via the natural lack of crossing over between homologous chromosomes in male *D. melanogaster*, and the use of specially constructed clone-generator (CG) females. CG females posses: (1) a compound X chromosome (*C(1)DX*, *y, f*) consisting of two X chromosomes linked at the centromere, and (2) two copies of a homozygous-viable translocation of the two major autosomes (*T(2;3) rdgC st in ri p*P *bw* ^{D}, see Figure I). CG females also have a Y chromosome (which must be sampled from the source population to be investigated), but are still female because sex determination is controlled by the X to autosome ratio in *Drosophila*. The combination of the Y and compound X chromosome in CG females causes the paternal X chromosome to be transmitted from father to son, and the autosomal translocation forces the two major paternal autosomes to be passed on together (otherwise aneuploidy results). By controlling transmission of the X and the two major autosomes (II and III), individuals that are identical across more than 99.5% of the genome can be produced.

To produce a single hemiclone, a male from the source population must be mated to a CG female (cross 1 in Figure I). This cross produces a number of sons possessing various haploid combinations of the paternal chromosomes, plus a Y chromosome and the translocated autosomes from the CG mother. A single one of these heterozygous sons is then selected for clonal amplification by mating him to a new CG female (cross 2). This cross results in the production of sons that are genetically identical for the wildtype paternal haploid genome. By crossing these sons to new CG females, many individuals with a clonally amplified haploid genome can be produced in a short time (crosses 3 to N). The amplified clonal genomes are then expressed in a random wildtype background for analysis. To produce hemiclonal females, the amplified clonal males are mated to wildtype females from the original source population (cross N+1). To produce males, the compound X must first be backcrossed into females from the source population. When these DX (double X) females are mated to clonally amplified males they produce hemiclonal sons (cross N+1). Note that for each cross a number of

genotypes other than the target are produced, but that these are either inviable or can be selected out on the basis of their phenotype.

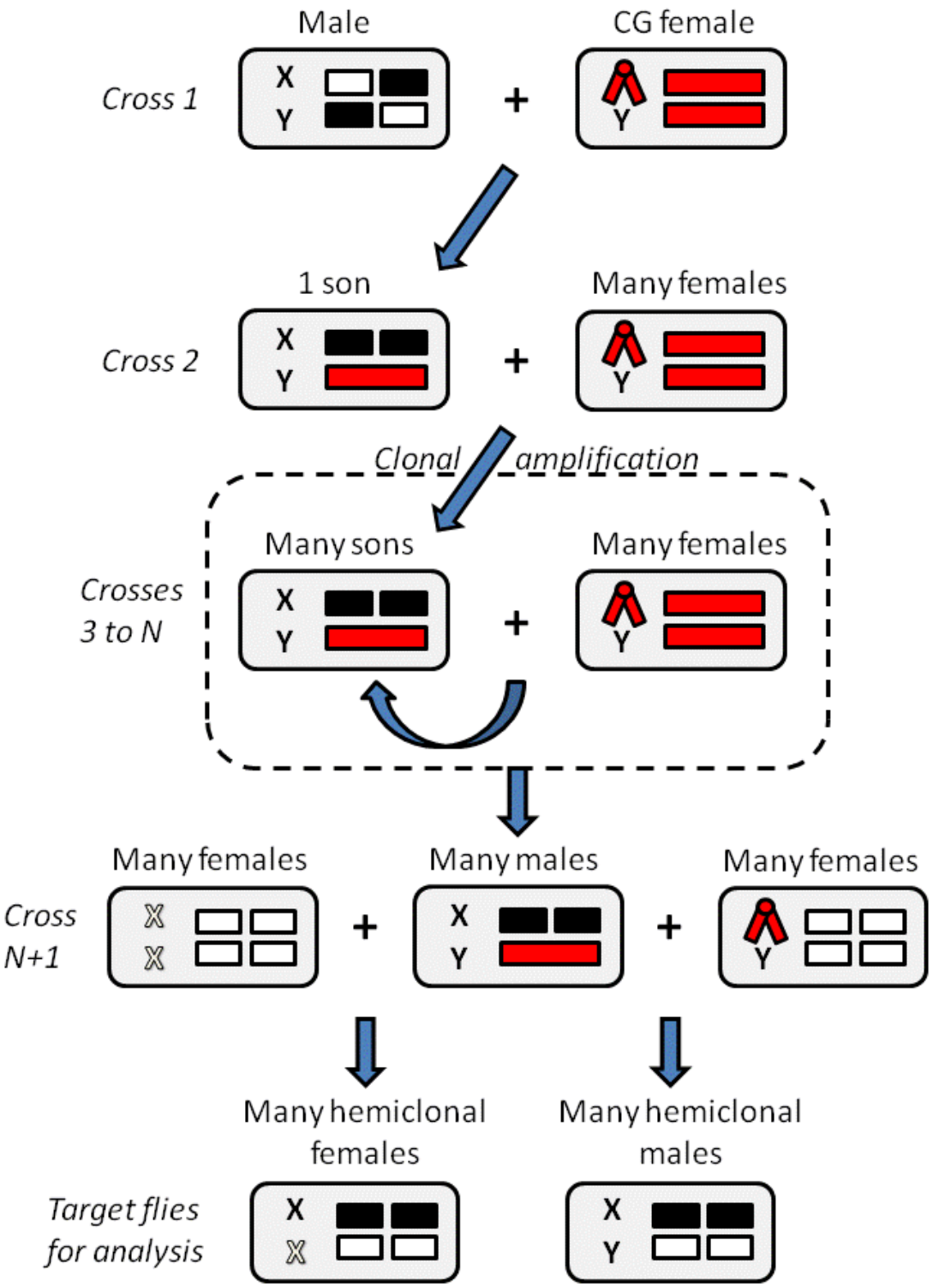
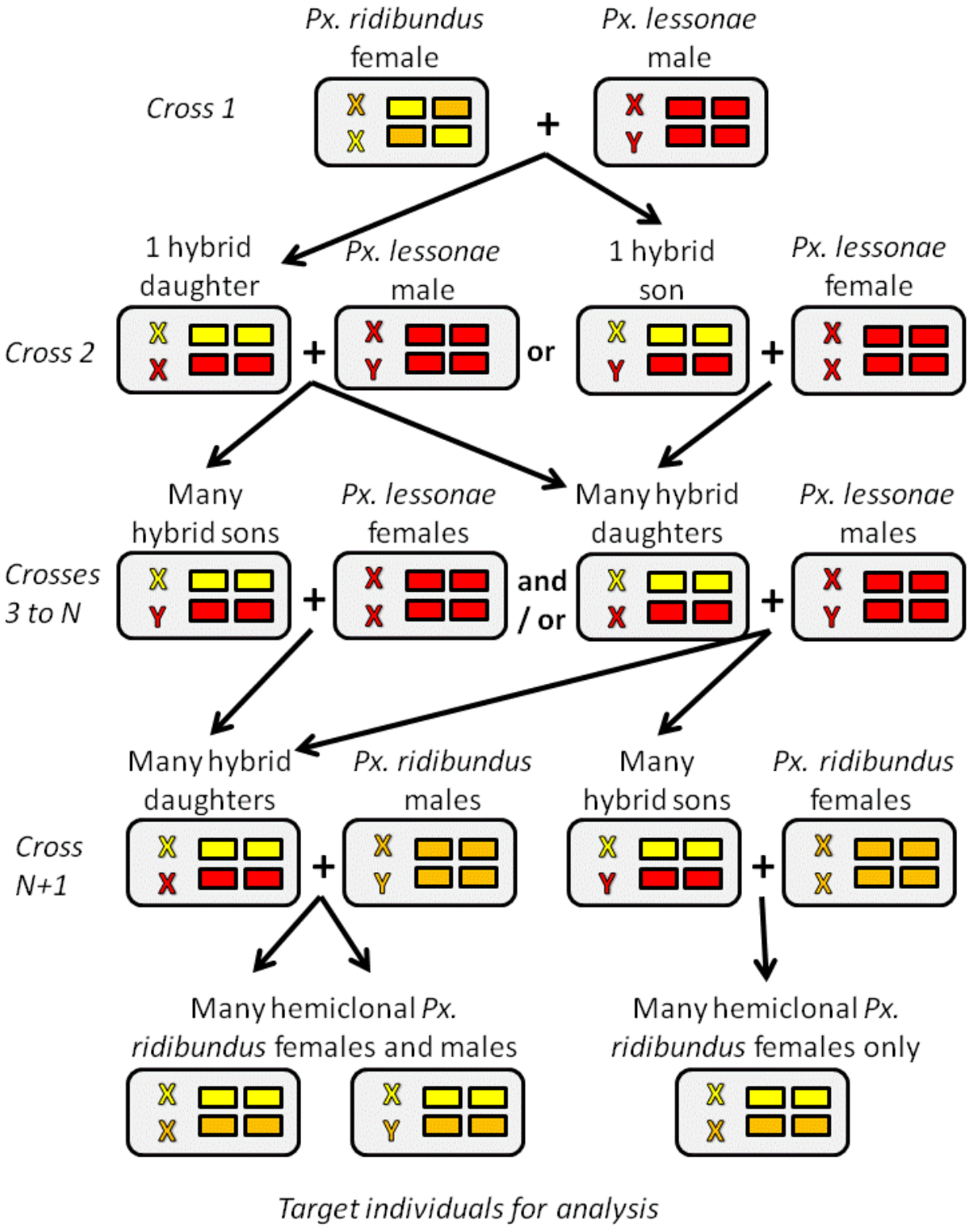


Figure I: Crossing scheme for the production of hemiclonal individuals. The compound X is represented by a red chevron, and the translocated autosomes by long red bars. Wildtype chromosomes from the source population are represented by short black and white bars. Figure is modified from Rice *et al*. 2005 [1]. Clone-generator (CG) females are first crossed to wildtype males. The male offspring of this cross will have one wildtype haploid genome and one CG genome. A single F1 male is then crossed to several new CG females, resulting in amplification of the wildtype genome. The amplified hemiclonal genome can then be expressed as either sex in a random genetic background for analysis. Note that CG females are taken anew from a separate stock population every generation.

Box 3: Detailed description of the production of *Px. ridibunda* hemiclones

In nature, individuals of *Px. esculentus* have one haploid genome from the parental species *Px. ridibundus* and one from the other parental species *Px. lessonae*. Synthetic *Px. esculentus* hemiclonal hybrids can be produced in the laboratory by crossing a female *Px. ridibundus* to a male *Px. lessonae*. This property can be exploited to produce *Px. ridibundus* hemiclones for studies of standing genetic variation.

To produce hemiclonal *Px. ridibundus* individuals, a synthetic hybrid must first be produced by crossing female *Px. ridibundus* and a male *Px. lessonae* (Figure I). After this, a single individual is selected for clonal amplification. This individual could be either male or female, although for different reasons one might be preferred over the other. For example, a hybrid male might be preferred because a single male can potentially fertilize several females, and this will speed up clonal amplification. However, crossing a hybrid male to a *Px. lessonae* female will result in the introduction of *Px. lessonae* mitochondrial DNA (mtDNA) to the clonal amplification line [14]. *Px. lessonae* mtDNA can later be removed from the male line by crossing males with *Px. lessonae* mtDNA to *Px. ridibundus* females (offspring will inherit the *Px. ridibundus* mtDNA), but cannot be removed from the female line once it has been introduced. Regardless of the sex of the hybrid individual it is always the *Px. ridibundus* X chromosome which is passed on to offspring, which means that female hybrids will produce offspring of both sexes, but male hybrids will only produce female offspring. After the initial clonal amplification cross, all individuals will share the same *Px. ridibundus* haplotype, and clonal amplification can be continued (using both males and females or only females) until the desired hemiclonal population size has been reached. At this point, hybrid individuals are backcrossed to *Px. ridibundus*, producing *Px. ridibundus* offspring with one hemiclonal genome and one random wildtype genome.



607

608 Figure I: Crossing scheme for the production of *Px. ridibundus* hemiclonal individuals. *Px. ridibundus*

609 chromosomes are in yellow and orange, *Px. lessonae* chromosomes are in red. For simplicity and for

610 ease of comparison with Box 1 Figure I, only the sex chromosomes and two autosomes are shown.

611 The final hemiclone production step can be carried out using either hybrid males or hybrid females,

612 but hybrid males will only produce daughters.

Figure 1: Simplified overview of how hemiclonal analysis allows for collection of "snapshots" of standing genetic variation. These hypothetical diploid organisms (rounded rectangles) have one chromosome (coloured bars), with different chromosomal colours representing different haplotypes. A number of individuals are randomly selected from the source population, and their haplotypes are amplified and expressed in a random genetic background. Each haplotype has its own hemiclonal line, and resulting in the production of many hemiclonal individuals that are genetically identical for a specific haplotype.

Figure 2: Crossing scheme for studying nuclear-mitochondrial interactions in *Pelophylax*. Different hemiclonal (nuclear) and mitochondrial genotypes are represented by numerical subscripts, and different mitochondrial genotypes and cytoplasmic factors are also represented by different colours. By carrying out reciprocal crosses between males and females from different synthetic hybrid hemiclonal lines, it should be possible to produce females with the same nuclear genotype (i.e. R_1R_2 in this example), but with different mitochondrial strains and cytoplasmic factors. These groups can then be compared to look for phenotypic effects of interactions between nuclear and mitochondrial genes.

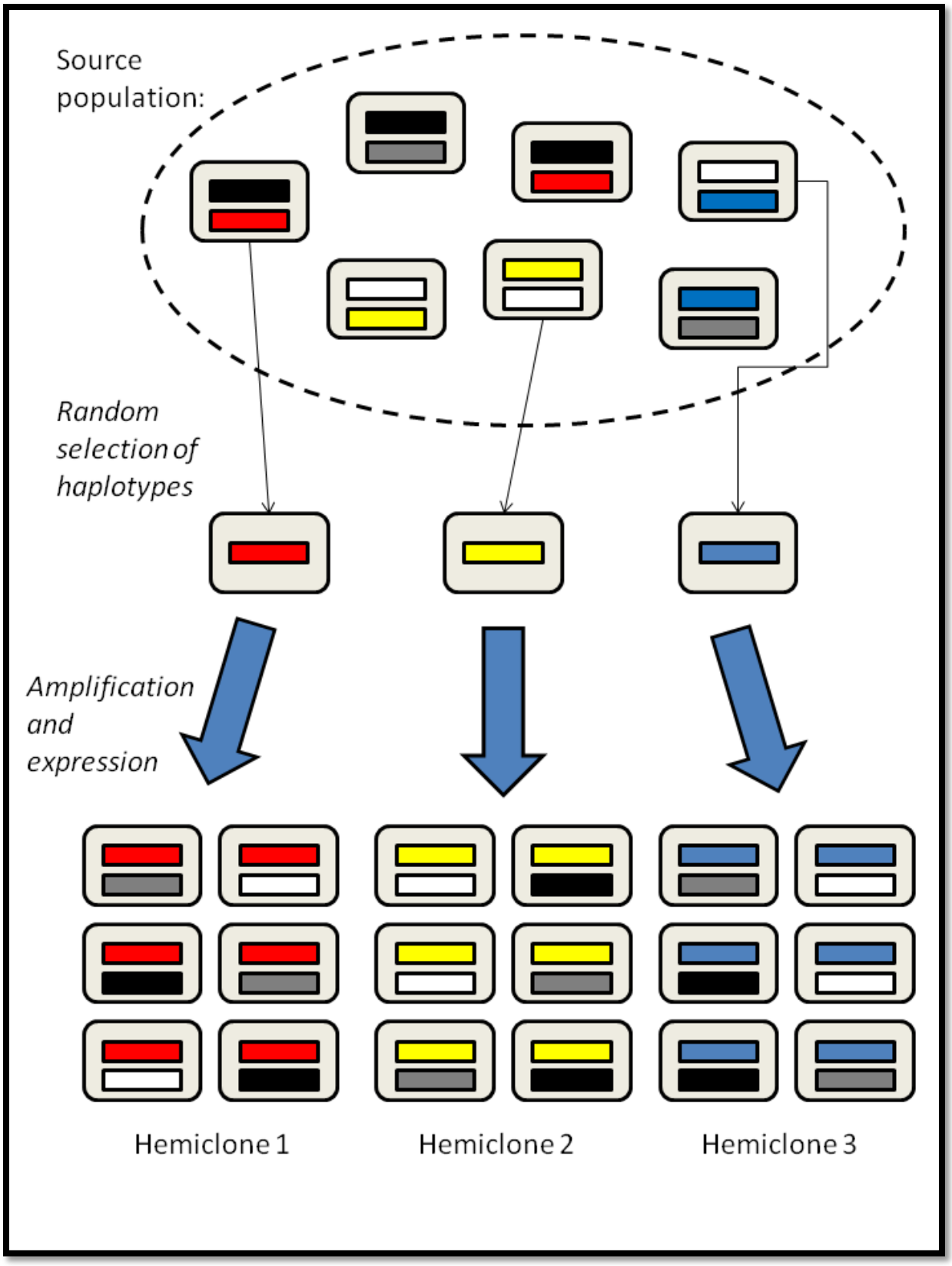
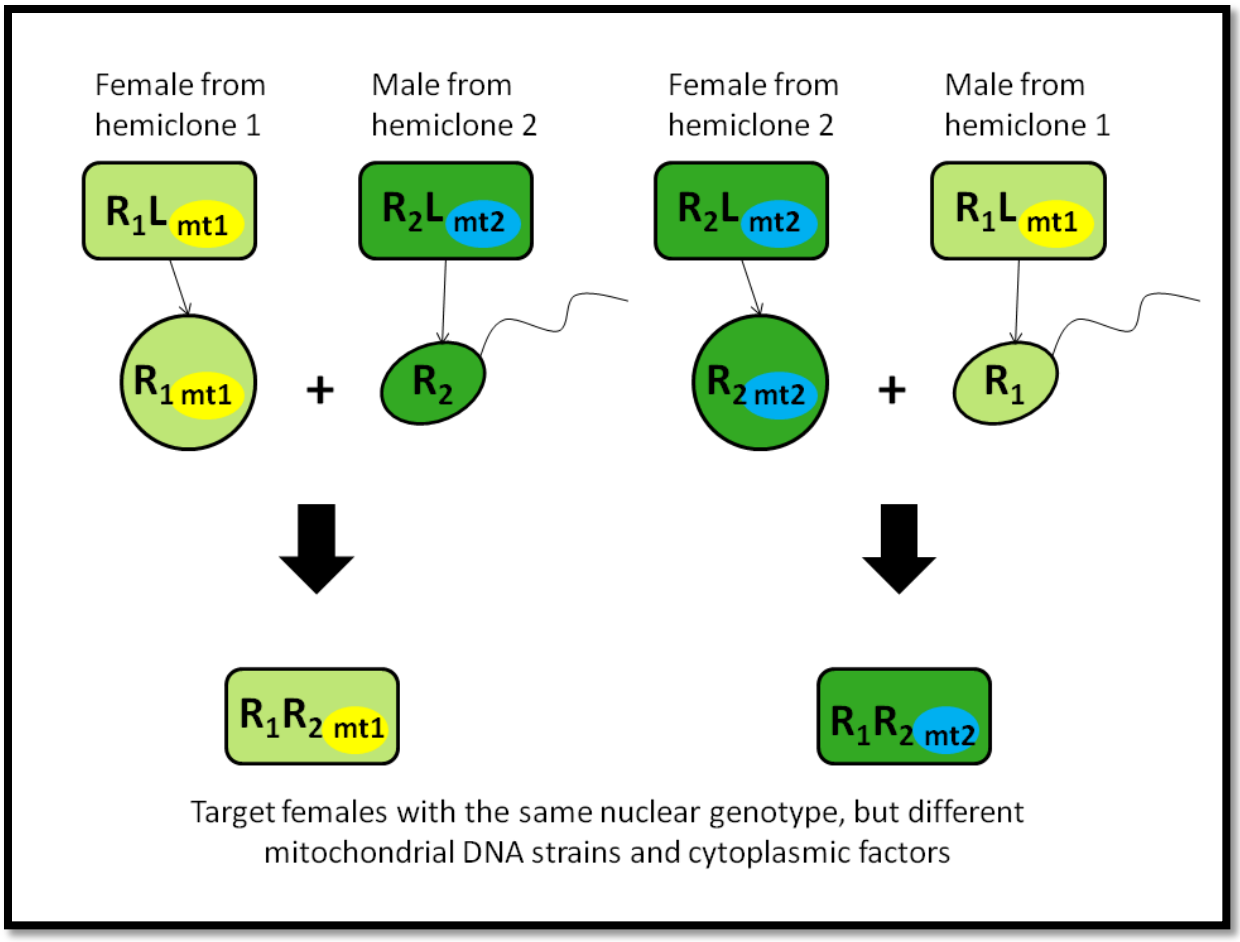

Source
population:
Random
selection of
haplotypes
Amplification
and
expression
Hemiclone 1
Hemiclone 2
Hemiclone 3

Figure 1



632

633 Figure 2

634

Glossary

Androgenesis: When maternal chromosomes are absent or inactivated in the egg, such that a zygote develops which contains only paternal genetic material.

Aneuploidy: Genetic abnormality where the number of chromosomes is not an exact multiple of the haploid number, such that some chromosomes (or parts of chromosomes) are missing or are present in extra copies. Usually lethal.

Balancer chromosome: A chromosome with multiple nested inversions carrying one or more phenotypic markers. Used to prevent recombination between homologous chromosomes during meiosis.

Breeding design: Standard crossing protocol for the estimation of quantitative genetic parameters, such as NCI (North Carolina Design I), or diallel.

Condition-dependence: When the expression of a trait depends on an individual's physical condition. Condition is determined via the interplay between environmental effects and a large number of genetic loci.

Diallel breeding design: Standard crossing protocol for the estimation of quantitative genetic parameters, where all female parents are crossed to all male parents.

Evolutionary lines of least resistance: When the genetic variance-covariance matrix is not uniform, such that an evolutionary response to selection is more likely in some phenotypic directions than in others.

Gametogenic parental species: Species whose genome is preserved and passed on clonally to offspring in natural hemiclonal hybrids. This species is usually the maternal parent in the original hybridization which produces the hemiclonal hybrids.

Gynogenesis: A form of female parthenogenesis in which the embryo contains only maternal chromosomes, but where the female still requires sperm to activate embryo development.

Hemiclone: A set of diploid individuals that share a single genetic haplotype. Hemiclones can be produced either naturally or artificially.

Hybridogenesis: When hybrid species reproduce via backcrossing to one of the parental species. Usually associated with hemiclonality or polyploidy.

Inbred line: A population of individuals of which are nearly identical to each other in genotype as a result of extensive inbreeding. Usually produced by repeated brother-sister matings over many generations.

Intralocus sexual conflict: When the same set of alleles have different fitness when expressed in males and females. For example if body size is controlled by the same loci in both sexes, but males are selected for small size and females for large size.

Interlocus sexual conflict: When males and females have conflicting interests over reproduction in traits that are controlled by different alleles. For example when there is a conflict over mating rates or parental investment.

Intersexual genetic correlation: A measure of the strength of the relationship between breeding values for a trait when expressed in males and females.

Lecithotrophy: When a developing embryo receives nutrition from the yolk of the egg.

Matrotrophy: When a developing embryo receives nutrition directly from the mother, for example via the placenta.

North Carolina Design I: Standard crossing protocol for the estimation of quantitative genetic parameters, where each male parent is mated to a different subset of female parents.

Quantitative trait locus (QTL): A polymorphic site on a chromosome containing alleles that differentially influence the expression of a quantitative trait.

Quantitative genetics: The study of phenotypic traits that are influenced by multiple genetic and environmental factors (i.e. polygenic traits).

Sexually antagonistic genetic variation: Genetic variation that has opposite fitness effects in males and females. Important in intralocus and interlocus sexual conflict.

Somatic parental species: Species whose genome is excluded from gamete production in natural hemiclonal hybrids. Hemiclonal individuals must therefore mate with this species to produce new hemiclonal offspring. This species is usually the paternal parent in the original hybridization which produces the hemiclonal hybrids.

Sperm offense: A male's performance in sperm competition when mated to a previously mated female.

Synthetic hybrid: Hemiclonal hybrids that are produced *de novo* in the laboratory by matings between the parental species.