

## REVIEW

**Androgenesis: a review through the study of the selfish shellfish *Corbicula* spp.**L-M Pigneur<sup>1</sup>, SM Hedtke<sup>2</sup>, E Etoundi<sup>1</sup> and K Van Doninck<sup>1</sup>

Among the asexual reproductive modes, androgenesis is probably one of the most astonishing and least studied mechanisms. In this 'paternal monopolization', the maternal nuclear genome fails to participate in zygote development and offspring are paternal nuclear clones. Obligate androgenesis is known in only a few organisms, including multiple species of clam in the genus *Corbicula*. *Corbicula* is a good system to review the evolutionary consequences of this 'all-male asexuality' because the cytological mechanisms of androgenetic reproduction have been described. In *Corbicula*, sperm are unreduced and, after fertilization, the maternal nuclear chromosomes are extruded as two polar bodies. Hermaphroditic lineages of *Corbicula* have a worldwide distribution and seem to reproduce through androgenesis, whereas their sexual relatives have restricted ranges. The invasive success of these androgenetic *Corbicula* lineages may be linked to their asexual mode of reproduction. We review the phenomenon of androgenesis, focusing on evolutionary perspectives, using the genus *Corbicula* as an exemplar system. *Heredity* (2012) **108**, 581–591; doi:10.1038/hdy.2012.3; published online 4 April 2012

**Keywords:** androgenesis; asexuality; *Corbicula* spp.; polyploidy; spermatogenesis

**INTRODUCTION**

Asexual reproduction in eukaryotes can be defined from either a life-cycle or genic perspective. From a life-cycle or cellular perspective, organisms that reproduce asexually do not cycle between ploidy levels (Bengtsson, 2009). From a genic perspective (which can be applied outside of eukaryotes), asexual reproduction occurs when offspring inherit a copy of one parent's genome unmodified by recombination. Most forms of asexuality—parthenogenesis, gynogenesis and androgenesis—would be classified as asexual under both definitions. Some parthenogenetic organisms, however, produce recombinant gametes through meiosis, and then ploidy is subsequently restored (reviewed in Haccou and Schneider, 2004). Even though there is only one parent, both recombination and alternation of ploidy between generations has occurred. Hybridogenesis would be classified as sexual under a cellular perspective, as females produce a haploid egg that is fertilized by a sperm, and the offspring contain both female and male genomes. However, in the next generation, only the female genome is transmitted to the haploid gametes. From a genic perspective, hybridogenetic species could be considered asexual. Asexuality is thus an unfortunate term because it seems to imply 'no sex'.

Asexuality evolved many times in the eukaryotic tree, and there are many different causes for the origin of asexuality among different groups. Bengtsson (2009) argued that there is no unified explanation for the evolution of asexuality and each case should be studied separately as an outcome of a Darwinian process with very specific properties, dependent on genetic and ecological factors. In this light, we review the asexual phenomenon of androgenesis. Androgenetic reproduction, also known as paternal apomixis (for example, Pichot *et al.*, 2008), 'maternal genome loss' (Burt and Trivers, 2006) or even

'all-male asexuality' (Hedtke *et al.*, 2008), has been described as a 'paternal monopolization of parenthood' (Normark, 2009) because the embryo contains only paternal nuclear chromosomes deriving from one or more sperm nuclei. Androgenesis has long been considered a rare phenomenon and has been recorded in relatively few organisms (McKone and Halpern, 2003; Hedtke and Hillis, 2011). Moreover, data on the mechanisms and the evolution of this odd reproductive mode in most organisms are scarce. Here we focus on the reproduction and possible evolutionary consequences of obligate androgenesis in one clade of the clam genus *Corbicula*.

**ANDROGENESIS: A RARE PHENOMENON**

Androgenesis is taxonomically widespread across the Tree of Life (Hedtke and Hillis, 2011), but appears to be relatively rare compared with all-female asexual reproduction. This comparative rarity could be due to androgenesis being rarely identified, or because androgenesis might be difficult to evolve or maintain once it has arisen (McKone and Halpern, 2003). In animals, androgenesis requires identification of parentage through either genetic or cytological studies, and may be overlooked in natural populations. Alternatively, there may be genetic constraints that prevent viable androgenetic offspring from developing, or mutations that lead to androgenesis may be unlikely or require a mutational pathway that would cause a temporary reduction in fitness. Once it does arise, obligate androgenesis could lead to extinction in species with two sexes (McKone and Halpern, 2003), or its spread could be halted if there is coevolution of females that prevent fertilization by androgenetic males. Three types of androgenesis have been distinguished (reviewed in Hedtke and Hillis, 2011). The first type, artificial androgenesis, is induced in the laboratory to

<sup>1</sup>Research Unit in Environmental and Evolutionary Biology (URBE), University of Namur (FUNDP), Namur, Belgium and <sup>2</sup>Section of Integrative Biology and Center for Computational Biology and Bioinformatics, University of Texas, Austin, TX, USA  
Correspondence: Dr L-M Pigneur, Research Unit in Environmental and Evolutionary Biology (URBE), University of Namur (FUNDP), Rue de Bruxelles 61, Namur 5000, Belgium.  
E-mail: Impigneur@fundp.ac.be

Received 17 September 2011; revised 8 January 2012; accepted 13 January 2012; published online 4 April 2012

produce progeny with only paternal nuclear genes. Artificially induced androgenesis is used in many teleost fishes where the maternal genome is destroyed and the embryo derives from a double haploid paternal genome (for example, Parsons and Thorgaard, 1984; May *et al.*, 1988) and in angiosperms where haploid microspores are induced by a stressor to develop into embryos (reviewed in Pandey, 1973).

The second type of androgenesis is spontaneous androgenesis. Here, organisms that usually reproduce sexually shift spontaneously to androgenetic reproduction, but at a relatively low frequency. Spontaneous androgenesis has been recorded in plant and animal phyla, including angiosperms, fishes and arthropods (reviewed in Normark, 2009; Hedtke and Hillis, 2011). The mechanisms for spontaneous androgenesis vary widely across these groups, and can sometimes be associated with other asexual characteristics. For example, stick insects of the genus *Bacillus* exhibit various reproductive modes, including sexual reproduction, facultative and obligate parthenogenesis, hybridogenesis and spontaneous androgenesis (Mantovani and Scali, 1992; Komma and Endow, 1995).

Obligate androgenesis is the third type of androgenesis with all progeny inheriting only the paternal nuclear genome. McKone and Halpern (2003) used simulations to explore the evolutionary implications for systems in which obligate androgenesis arises. They argue that alleles for obligate androgenesis are expected to be advantageous to those males that carry them, and will spread once they arise in the populations. Under many of their model conditions, this increase in frequency occurs at the expense of the population: androgenesis could decrease population mean fitness. The spread of androgenesis can even lead to extinction if it causes loss of females in dioecious species, especially if androgenesis results from paternal apomixis (fusion or doubling of haploid nuclei) and if females are the heterogametic sex. Thus, one important conclusion of McKone and Halpern's (2003) work is the importance of hermaphroditism in the evolutionary maintenance of androgenesis. McKone and Halpern (2003) discuss three hypotheses as to why obligate androgenesis seems rarer than its 'female' corollary: (i) It could be more difficult to detect androgenesis than all-female asexuality because the latter can be identified when a female reproduces in the absence of male gametes. (ii) Alleles for androgenesis and all-female asexuality could arise at similar frequencies, but androgenetic lineages could be lost more often through extinction. In several of the model scenarios explored by McKone and Halpern (2003), extinction occurs directly after the fixation of androgenetic alleles. (iii) The mutations to produce all-female parthenogenesis could be more frequent than mutations that produce androgenesis. These latter could require a more complex set

of mutations in multiple genes, a more complex mutational pathway within a gene or arise in unusual cases only.

Until now, obligate androgenesis is known in only the following organisms (Table 1): the Tassili cypress tree *Cupressus dupreziana* (Pichot *et al.*, 2001), the little fire ant *Wasmannia auropunctata* (Fournier *et al.*, 2005) and several species of the clam genus *Corbicula* (Komaru *et al.*, 1998; Ishibashi *et al.*, 2003). The mechanisms of androgenesis differ widely across these three obligately androgenetic lineages.

The Tassili cypress tree, *Cupressus dupreziana*, is a very rare species restricted to a small area of Sahara Desert in Algeria (Pichot *et al.*, 2001; Abdoun and Beddiaf, 2002). This species produces unreduced, diploid pollen, presumably due to aberrant meiosis of microspores (Pichot and El Maâtaoui, 2000). The father is the sole source of nuclear genes in the embryo (as shown by the lack of electrophoretic alleles from the mother) (Pichot *et al.*, 2000, 2001; El Maâtaoui and Pichot, 2001). However, only 10% of the seeds of *C. dupreziana* contain a viable embryo, probably in part because of the high proportion of empty ovules (Pichot *et al.*, 1998), and this species is critically endangered. The mechanism for obligate androgenesis in this cypress tree differs from the mechanisms in androgenetic animals. Instead, many (or possibly all) ovules do not contain egg cells, and embryogenesis appears to occur directly from the pollen (Pichot *et al.*, 1998; Pichot *et al.*, 2008). Several researchers have speculated about whether androgenesis could have been selected for as a response to small population size. Pichot *et al.* (2001) suggested that androgenesis could be considered an evolutionary response that prevents inbreeding depression due to uniparental inheritance, whereas Burt and Trivers (2006) argued that the main advantage is in the ability of *C. dupreziana* to use ovules of the closely related species *C. sempervirens*, which then acts as a 'surrogate mother' (Pichot *et al.*, 2001). However, while this latter phenomenon was demonstrated in greenhouse experiments, there is no evidence that it is effectively occurring in nature.

The second case of obligate androgenesis is the 'sex-limited' androgenesis in the haplodiploid system of the little fire ant, *Wasmannia auropunctata* (Fournier *et al.*, 2005). Diploid queens are produced by apomictic parthenogenesis, whereas sterile workers are sexually produced. Haploid males are produced by androgenesis. The nuclear genome of a male is inherited completely from the sperm nucleus of his father. From a life cycle and a genic perspective, both male and female reproductives are independently reproducing asexually. As a consequence, male and female genetic pools are completely separated (Fournier *et al.*, 2005; but see Foucaud *et al.* (2006) for evidence for rare sexual reproduction).

**Table 1** Organisms reproducing through obligate androgenesis (modified from McKone and Halpern (2003) and Hedtke and Hillis (2011))

Organism	Hermaphrodite/dioecious	Evidence	Reference
<i>Angiosperms</i>			
<i>Cupressus dupreziana</i>	Hermaphrodite	Paternity	Pichot <i>et al.</i> (2001)
<i>Molluscs (bivalves)</i>			
<i>Corbicula leana</i>	Hermaphrodite	Cytology and morphology (biflagellate sperm)	Komaru <i>et al.</i> (1998)
<i>Corbicula fluminea</i>	Hermaphrodite	Cytology and morphology (biflagellate sperm)	Ishibashi <i>et al.</i> (2003)
<i>Corbicula australis</i>	Hermaphrodite	Morphology (biflagellate sperm)	Byrne <i>et al.</i> (2000)
<i>Corbicula fluminalis</i>	Hermaphrodite	Morphology (biflagellate sperm)	Korniushin (2004)
<i>Arthropods (insects)</i>			
<i>Wasmannia auropunctata</i> (haploid males)	Dioecious (haplodiploidy)	Paternity	Fournier <i>et al.</i> (2005)

The third identified case of obligate androgenesis is in the clam genus *Corbicula*. *Corbicula* is found globally in fresh and brackish waters, and includes both dioecious sexual species and hermaphroditic androgenetic species (Table 2). Androgenesis in *Corbicula* occurs in lineages with the derived characteristics of hermaphroditism and unreduced biflagellate sperm (that is, sperm containing the same number of nuclear chromosomes as somatic cells), as opposed to dioecious sexuals with uniflagellate reduced sperm (for example, Ishibashi *et al.*, 2003; Table 2). In androgenetic *Corbicula*, cytological studies show that the oocyte is fertilized by an unreduced sperm. The entire maternal nuclear genome is then extruded from the oocyte as two polar bodies, whereas mitochondria and other organelles from the egg are retained (Figure 1). Thus, the offspring inherit only paternal nuclear chromosomes and are clones of their father. Of all obligately androgenetic species identified, the cytological mechanisms are best understood in *Corbicula*. Thus, *Corbicula* remains the best system to explore the consequences of obligate androgenesis.

## THE SELFISH SHELLFISH: REPRODUCTIVE PECULIARITIES OF THE GENUS *CORBICULA*

### Reproductive characteristics

Most hermaphroditic bivalves are protandrous (that is, spermatozoa are produced in the life cycle before eggs) (Kraemer *et al.*, 1986). However, hermaphroditic species of *Corbicula* are protogynous: female gametes are produced before the sperm. In North American *Corbicula*, eggs are present year-round and sperm appear to be produced in response to seasonal changes in temperature (Kraemer *et al.*, 1986). Cross-fertilization between hermaphroditic individuals is suggested by the presence of mucous filaments containing sperm connecting their siphons (Kraemer *et al.*, 1986). Self-fertilization has also been suggested in *Corbicula* (Ikematsu and Yamane, 1977; Kraemer *et al.*, 1986): embryos were found in the visceral mass, within the gametogenic follicles, whereas most embryos are usually incubated in the gills in hermaphrodites (for example, Glaubrecht *et al.*, 2006). Recently, Houki *et al.* (2011) found a Japanese population of *C. leana* that contains both males and hermaphroditic individuals. The cytological and genetic results suggest androdioecy, with androgenetic males fertilizing eggs of hermaphrodites.

For freshwater bivalves, the dioecious, free-spawning and dispersive life history is considered the primitive condition (Kraemer and Galloway, 1986). In the genus *Corbicula*, the lacustrine species *C. sandai* and the brackish water species *C. japonica* share these 'primitive' life-history traits (Okamoto and Arimoto, 1986; Konishi *et al.*, 1998; Korniusshin and Glaubrecht, 2003). However, lacustrine species of *Corbicula* from Sulawesi and Sumatra in Indonesia seem dioecious and sexual, but incubate their larvae (Glaubrecht *et al.*, 2003). All other freshwater *Corbicula* clams are hermaphroditic and brood their larvae in their gills (Komaru *et al.*, 1997; Byrne *et al.*, 2000; Qiu *et al.*, 2001; Glaubrecht *et al.*, 2006).

### Androgenetic sperm

Within the bivalves, *Corbicula* is the only known genus exhibiting biflagellate unreduced sperm in some species, whereas others produce monoflagellate, reduced sperm (Table 2 and Figure 3). Biflagellate unreduced sperm is only observed in androgenetic hermaphroditic *Corbicula* species (Table 2). Recently, males of *C. leana* coexisting with hermaphroditic androgenetic individuals were also found to produce biflagellate unreduced sperm (Houki *et al.*, 2011). Most bivalves,

however, have monoflagellate reduced sperm (the primitive or ancestral form). In *Corbicula* aff. *fluminea* from Japan, Konishi *et al.* (1998) observed spermatozoa with a rod-like acrosome, an indistinct mid-piece, two long flagella and an elongated head. In bivalves, the presence of an acrosomal structure is usually associated with brooding (Franzén, 1983), although the non-brooding *C. sandai* also has a prominent acrosome (Konishi *et al.*, 1998). Spermatozoa with elongated heads are correlated with fertilization of large, yolk-rich eggs (Franzén, 1983). Within the genus *Corbicula*, biflagellate sperm are unique to androgenetic lineages (including males coexisting with androgens), as far as is known. Moreover, the situation of androdioecy, found recently in *C. leana* (Houki *et al.*, 2011), is very rare in the animal kingdom (Weeks *et al.*, 2006).

Several hermaphroditic freshwater species of *Corbicula* produce unreduced spermatozoa: that is, sperm cells containing the same amount of DNA as somatic cells (Komaru *et al.*, 1997, Houki *et al.*, 2011; see Table 2). On the other hand, the dioecious, sexually reproducing *Corbicula* species produce reduced sperm: the DNA content of spermatozoa is half of that of somatic cells. Ishibashi *et al.* (2003) concluded that, in *Corbicula*, unreduced biflagellate sperm could be considered indicative of androgenesis. The presence of biflagellate sperm in the invasive lineages in the Americas (Lee *et al.*, 2005; Table 2 and Figure 3) and Europe (Pigneur *et al.*, 2011b; Table 2 and Figure 3) suggests that androgenesis is the most likely reproductive mode in invasive *Corbicula*.

To date, few studies describe spermatogenesis in *Corbicula*, and the mechanism that causes unreduced sperm still remains unknown. By analogy to the production of unreduced oocytes in parthenogens, the production of unreduced spermatozoa could occur through apomixis (that is, without meiosis) or through automixis (that is, with meiosis) (Maynard Smith, 1978; McKone and Halpern, 2003). Apomictic production of diploid (or polyploid) sperm derives from mutations in the meiotic mechanism that arise quite frequently in animals (reviewed in McKone and Halpern, 2003). Automictic production of unreduced sperm could occur through several proposed mechanisms, and each of these will have different expectations for the genetic consequences: (i) pre-meiotic doubling of DNA during spermatogenesis (Komma and Endow, 1995; Haccou and Schneider, 2004); (ii) fusion of sperm after meiosis (McKone and Halpern, 2003) and (iii) abortive cytokinesis during meiosis (Komaru *et al.*, 1997). Any viable hypothesis for the production of unreduced sperm in *Corbicula* must consider both the maintenance of not just diploid, but triploid (and maybe tetraploid) sperm, and the observation of heterozygosity across nuclear loci (Hedtke *et al.*, 2008; Pigneur *et al.*, 2011b). Both apomixis and pre-meiotic doubling of the chromosomes would result in the maintenance of heterozygosity over time (Haccou and Schneider, 2004). Fusion of sperm after meiosis and abortive cytokinesis during meiosis II would, over time, reduce individual heterozygosity. In *Corbicula*, only one male pronucleus is directly observed after fertilization of the egg cell (Komaru *et al.*, 1998), whereas, in some insects, fused sperm with two flagella show two acrosomes and two nuclei (Dallai *et al.*, 2001). This suggests that fusion of sperm seems not the mechanism for unreduced sperm production in those androgenetic clams.

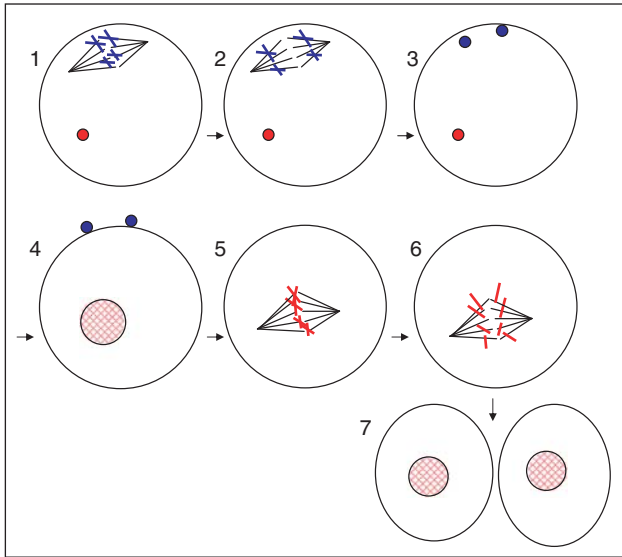
After recombination and segregation, a heterozygous male at a locus would produce both heterozygous and homozygous spermatozoa, whereas a homozygous male would only produce homozygous spermatozoa (barring any new mutations). Further examination of spermatogenesis will be a critical next step in determining the genetic consequences of androgenesis, as sperm production is the time in the

Table 2 Biological features of *Corbicula* species

Species	Chromosome number	Ploidy	Number of sperm flagella	Sperm ploidy	Sexuality (evidenced by cytology)	Diocy/monoocy	Brooding of larvae	Swimming larvae	Habitat	Locality	References (see legend)
<i>C. japonica</i>	38	2		Reduced	Sexual	Di	No	Yes	BW	Japan, China	1, 2
<i>C. sandai</i>	36	2	1	Reduced	Sexual	Di	No	No	FW	Japan (Lake Biwa)	1
<i>C. fluminea</i>	54	3	2	Unreduced	Androgenesis	Di/mono	Yes	No	FW	Korea	3
<i>C. fluminea</i>	36	2	2	Unreduced	Androgenesis				FW	Japan (invasive)	4-6
<i>C. fluminea</i> Müller (yellow morph)	54	3	2	Unreduced		Mono	Yes		FW	China	7
<i>C. fluminea</i> Müller (brown morph)	72	4	2	Unreduced		Mono	Yes		FW	China	7
<i>C. papyracea</i>	54	3							FW	Korea	3
<i>C. colorata</i>	38	2							FW	Korea	3
<i>C. leana</i>	54	3	2	Unreduced	Androgenesis	Mono	Yes	Yes	FW	Japan	1, 4
<i>C. australis</i>			2			Mono	Yes	Yes	FW	Australia	8, 9
<i>C. javanica</i>							Yes		FW	Indonesia	10
<i>C. moltkiana</i>			1				Yes	Yes	FW	Sumatra (lake)	10, 11
<i>C. linduensis</i>			1			Di	Yes	No	FW	Sulawesi (river)	10, 11
<i>C. matanrensis</i>			1			Di	Yes	No	FW	Sulawesi (lake)	10, 11
<i>C. loehensis</i>			1			Di	Yes	No	FW	Sulawesi (lake)	10, 11
<i>C. possoensis</i>			1			Di	Yes		FW	Sulawesi (lake)	10
<i>Form A</i>	54	3	2			Mono	Yes	No	FW	USA	8, 12
<i>Form B</i>	54	3	2			Mono	Yes	No	FW	USA	8, 12
<i>C. fluminalis</i>			2			Mono	Yes	No	FW	W/Central Asia, N. Africa	2
<i>C. madagascariensis</i>							Yes	No	FW	Madagascar	13
<i>Form R</i>	36	2							FW	Rhine, Germany	14
<i>Form S</i>	36	2							FW	Rhine, Germany	14
<i>Form S</i>	54	3							FW	Poland	15
<i>Form R</i>			2						FW	Several European rivers	16
<i>Form S</i>			2						FW	Several European rivers	16

For habitat: FW, fresh water; BW, brackish water habitats.

References: 1. Okamoto and Arimoto (1986); 2. Kornushin (2004); 3. Park *et al.* (2000); 4. Komaru *et al.* (1997); 5. Ishibashi *et al.* (2003); 6. Konishi *et al.* (1998); 7. Qiu *et al.* (2001); 8. Lee *et al.* (2005); 9. Byrne *et al.* (2000); 10. Glaubrecht *et al.* (2003); 11. Kornushin and Glaubrecht (2003); 12. Hedtke *et al.* (2008); 13. Glaubrecht *et al.* (2006); 14. Pfenninger *et al.* (2002); 15. Skuza *et al.* (2009); 16. Pigneur *et al.* (2011b).



**Figure 1** Schematic representation of the main steps of androgenetic reproduction in *Corbicula* spp. after fertilization. (1) The maternal chromosomes (in blue) are in metaphase I. The meiotic axis is parallel to the cell cortex. The male pronucleus is indicated in red. (2) The egg is in anaphase I and the maternal chromosomes segregate. (3) The cytoplasm around the asters is distorting and two polar bodies, containing all maternal chromosomes, are formed. (4) The male pronucleus expands. (The male gamete is considered unreduced, here diploid.) (5) Metaphase of the first cleavage (mitosis) of the zygote (with only 'paternal' chromosomes). (6) Anaphase of first cleavage. (7) Two-cell stage of the zygote. This schematic representation is based on cytological observations in Komaru *et al.* (1998).

life cycle of androgenetic *Corbicula* where recombination or segregation could potentially occur.

### FERTILIZATION AND MATERNAL MEIOSIS IN ANDROGENETIC *CORBICULA*

Komaru *et al.* (1997) initially hypothesized that *C. leana*, *C. fluminea* and *C. aff. fluminea*, three species with unreduced sperm, reproduced through pseudogamous gynogenesis. The maternal gametes would also be unreduced and sperm would activate development of eggs, but not contribute to offspring development. Gynogenesis would then explain the odd number of chromosomes found in *C. leana* ( $3n=54$ ), because gynogenetic eggs are usually meiotically unreduced and chromosome number is restored to the eggs by premeiotic endomitosis or abortive cytokinesis (Okamoto and Arimoto, 1986). Triploid *C. leana* would have evolved from a diploid ancestor following fusion of diploid and haploid gametes. Cytological observations of fertilized oocytes of *C. leana* indicated another mode of reproduction, androgenesis. We will detail this phenomenon here for *C. leana* after a brief introduction to meiosis.

In many invertebrates, the oocyte is arrested in meiosis I until fertilization; at this point, there are four haploid copies of the nuclear genome (reviewed in Sagata, 1996). Fertilization triggers the pursuance of meiosis I and induces a reorientation of the meiotic axis in the oocyte so that it is perpendicular to the egg surface. Ultimately, this reorientation causes the extrusion of half of the DNA adjacent to the egg cortex as a polar body. The remaining DNA progresses through meiosis II, and another half of the DNA is extruded as another polar body, leaving one haploid maternal set to fuse with the paternal nuclear chromosomes from the sperm. In most animals, including the

dioecious sexual species *C. sandai*, the meiotic axis is observed to be perpendicular to the egg surface (Komaru *et al.*, 1998).

Cytological observations of androgenetic *C. leana* revealed that the oocyte is also arrested at meiotic metaphase I until fertilization. In androgenetic *C. leana* and *C. fluminea*, however, the axis of the meiotic spindle observed in the oocyte is parallel to the egg surface after fertilization, while the nucleus of the spermatozoon condenses and forms a single male pronucleus (Komaru *et al.*, 1998). Owing to the peculiar orientation of the spindle, all maternal chromosomes are expelled (see Figure 1 for the detailed mechanism of zygote development). In *C. leana* and *C. fluminea*, the oocyte never goes through meiosis II, as the entire maternal nuclear genome is extruded during the first round of meiosis, and thus the reproductive mode is androgenetic (Komaru *et al.*, 1998; Ishibashi *et al.*, 2003).

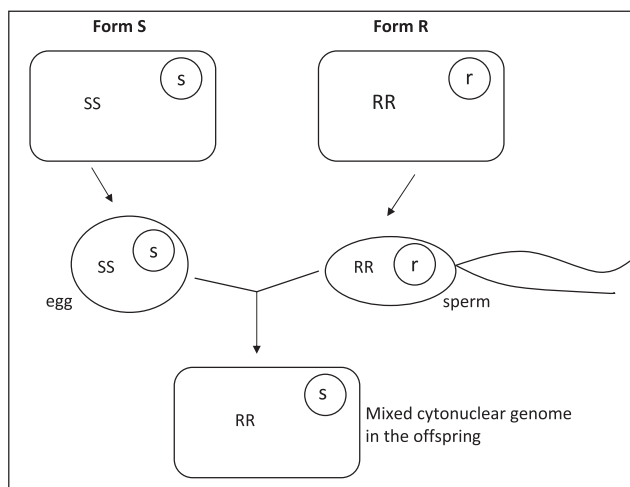
Komaru *et al.* (1998) hypothesized that this unusual phenomenon of axis orientation and complete expulsion of maternal chromosomes in androgenetic *Corbicula* would result from a 'meiosis-specific mutation', leading to the alteration of the orientation of the axis of the meiotic spindle. Furthermore, Komaru *et al.* (2000) suggested that androgenesis could also be caused by an alteration in the attachment site of the centrosomes. Dioecious species such as *C. sandai* have one attachment site, whereas the androgenetic *C. fluminea* has at least two attachment sites. To examine whether the oocytes of androgenetic *Corbicula* still have the capacity to go through meiosis II, several researchers treated fertilized eggs with cytochalasin D, a chemical known to inhibit polar body formation by retaining the meiotic centrosomes (formed by spindle fiber apparatus) and the maternal chromosomes. Ishibashi *et al.* (2002) treated eggs of triploid androgenetic *C. leana* with cytochalasin D and observed that the chromosomes and centrosomes are retained and the second meiosis occurs normally. Both maternal and paternal centrosomes occur (although the paternal centrosome remains inactive). These processes observed in cytochalasin D-treated eggs of triploid *C. leana* suggest that the system regulating second meiosis is still active. In contrast, Ishibashi and Komaru (2006) showed that the second meiosis is abortive in cytochalasin D-treated eggs of diploid androgenetic *C. fluminea*. At metaphase II, the chromosomes did not segregate. Apparently, some of the factors regulating second meiosis have become inactive in diploid *C. fluminea*. In obligately androgenetic species, factors for meiosis II are not essential and can become inactive because of relaxed selection for functional segregation of chromosomes at meiosis II. Over the long term, this would lead to an accumulation of mutations on the genes controlling meiosis II.

In *Corbicula*, a mutation in the ability of the oocyte to correctly orient the spindles at first meiosis, or a mutation in the signal sent from the sperm to orient the spindles correctly, may be the underlying cause of their androgenetic reproduction (Hedtke *et al.*, 2008). Either mutation would cause the maternal genome to be ejected during meiosis I. Subsequent mutations could cause the loss of function in meiosis II, as has already occurred in *C. fluminea* (Ishibashi and Komaru, 2006). Animals do not seem to be as tolerant to haploidy as plants are (Otto and Goldstein, 1992), but in *Corbicula*, as the spermatozoa are unreduced, the zygote develops with the same ploidy as the parent. We would speculate that this further suggests that unreduced sperm either evolved before the loss of correct orientation of the axis of meiotic spindle or that the underlying cause is the same: that the failure of the spindles to align correctly during gametogenesis could cause unreduced sperm in the male function and loss of maternal genome during female function. To understand the evolutionary steps in the origin of androgenesis in *Corbicula*, it is critical to understand the mechanisms that underlie spermatogenesis.

### MITOCHONDRIAL GENOME CAPTURE AND CYTOPLASMIC–NUCLEAR PHYLOGENETIC DISCORDANCE

Although nuclear chromosomes of androgenetic offspring come from the father, the mitochondria are still inherited maternally in *Corbicula* (Stepien *et al.*, 1999). As androgenetic *Corbicula* are hermaphroditic and potentially self-fertilizing (Kraemer *et al.*, 1986), most of the time this distinction is meaningless in the offspring. However, if sperm of one androgenetic lineage can fertilize the egg of a second lineage, this can lead to interesting evolutionary and phylogenetic consequences (Hedtke and Hillis, 2011). The maternal nuclear genome of the second lineage will be expelled from the egg, whereas the nuclear genome of the father develops and the maternal cytoplasmic genome is captured (for example, Lee *et al.*, 2005; Figure 2). The ability of androgenetic individuals of one lineage to ‘parasitize’ the maternal gametes of another lineage could enhance their reproductive fitness and their invasion success compared with sexual individuals.

Empirical evidences suggest that androgenetic individuals are able to parasitize other lineages. The signature of such an androgenetic event between lineages is incongruence between the mitochondrial haplotype of that lineage and its phenotype or nuclear genotype (Table 3 and Figure 3). In general, individuals of the same morphotype across androgenetic *Corbicula* share the same mitochondrial and nuclear haplotypes (Park and Kim, 2003; Lee *et al.*, 2005; Hedtke *et al.*, 2008; Pigneur *et al.*, 2011a,b). However, mitochondrial lineages discordant with morphology have been reported in several populations of *Corbicula* in Asia, America and Europe (Park *et al.*, 2002; Pfenninger *et al.*, 2002; Lee *et al.*, 2005; Hedtke *et al.*, 2008; Pigneur *et al.*, 2011b; see Figure 3). For example, invasive forms in the Americas and Europe are fixed across most of their range for unique mitochondrial haplotypes, but, in some populations, individuals of one morphological form harbor the mitochondrial *COI* sequence of the other form (for example, between forms A and B, B and C or R and S; Pfenninger *et al.*, 2002; Lee *et al.*, 2005; Hedtke *et al.*, 2008; Pigneur *et al.*, 2011b). In those cases, the mitochondria of one lineage are found associated with the nuclear genome of a second lineage. Egg parasitism during androgenesis leads to such cytoplasmic–nuclear



**Figure 2** Schematic illustration of mitochondrial genome capture and fixation of mixed cytonuclear genome in the case of the European *Corbicula* lineages forms R and S (Hedtke and Hillis, 2011; Pigneur *et al.*, 2011b). The ploidy of forms R and S is still uncertain ( $2n$  or  $3n$ ), but the general mechanism of mitochondrial capture remains the same in the different cases. Here, the egg is considered diploid (unreduced) as it is in metaphase I 15 min after spawning and the reductional division has not occurred yet.

discordance in only one generation (Figure 2). The mixed ‘cytonuclear genotype’ can rapidly spread and become fixed in a population (Hedtke and Hillis, 2011), especially if selfing occurs. Two invasive *Corbicula* lineages (forms A and B) co-occur in Texas, and at least one river drainage has become fixed for the mitochondrial haplotype of the other lineage in only 30 years (Hedtke *et al.*, 2008).

Mitochondrial phylogenies have revealed that the androgenetic clams of the genus *Corbicula* are polyphyletic, including 3 or 4 clades

**Table 3** GenBank accession numbers, *COI* haplotype designation and localities of *Corbicula* spp. sequences included in the phylogenetic analysis

Taxa and haplotype code	Origin	GenBank accession numbers
<i>C. sandai</i> A	Japan	AF196272
<i>C. sandai</i> B	Japan	AF196273
<i>C. australis</i>	Australia	AF196274
<i>C. fluminea</i> , <i>C. sp. form B</i> (FW1)	Korea/America	AF196269, AF519509–11
<i>C. sp. form C like</i> (C2), Iguazu Falls	Argentina	AF519512
<i>C. sp.</i> (FW2)	China	AF457989
<i>C. sp.</i> (FW3)	China	AF457990
<i>C. subplanata</i> , <i>C. sp. form R1c</i> (FW4)	Indonesia/France	GU721084, DQ285602
<i>C. linduensis</i>	Indonesia	DQ285579
<i>C. leana</i> , <i>C. sp. form A–form R</i> (FW5)	Japan/America–Europe	AF519495–507, GU721082, AF196268
<i>C. fluminea</i> (FW7)	France	AF269094
<i>C. sp.</i> (FW8)	Taiwan	AF457991
<i>C. javanica</i> (FW9)	Indonesia	AF457993
<i>C. sp.</i> (FW10)	Korea	AF457992
<i>C. sp.</i> (FW11)	China	AF457994
<i>C. sp.</i> (FW12)	China	AF457995
<i>C. sp.</i> (FW13)	China	AF457999
<i>C. fluminea</i> (FW14)	Thailand	AF196270
<i>C. sp.</i> (FW15)	Vietnam	AF468017
<i>C. sp.</i> (FW16)	Vietnam	AF468018
<i>C. sp. form C–form S</i> (FW17)	Argentina–Europe	GU721083, AF519508
<i>C. cf. japonica</i> ( <i>C. fluminalis</i> A)	China	AF457996
<i>C. cf. japonica</i> ( <i>C. fluminalis</i> C)	China	AF457998
<i>C. japonica</i> A	Japan	AF196271
<i>C. cf. japonica</i> (Kor1)	Korea	EU090396
<i>C. japonica</i> (KR1)	Korea	AF367440
<i>C. japonica</i> (KR2)	Korea	AF367441
<i>C. lamarckiana</i>	Thailand	DQ285578
<i>C. loehensis</i> 1	Indonesia	DQ285580
<i>C. loehensis</i> 2	Indonesia	DQ285581
<i>C. madagascariensis</i>	Madagascar	AF196275
<i>C. matannensis</i> 1	Indonesia	DQ285589
<i>C. matannensis</i> 2	Indonesia	AY275663
<i>C. matannensis</i> 3	Indonesia	AY275665
<i>C. matannensis</i> 4	Indonesia	DQ285592
<i>C. matannensis</i> 5	Indonesia	DQ295587
<i>C. matannensis</i> 6	Indonesia	DQ285594
<i>C. anomioides</i>	Indonesia	DQ285605
<i>C. possoensis</i>	Indonesia	DQ285598

The phylogenetic tree is presented in Figure 3.



**Figure 3** Mitochondrial phylogeny (maximum-likelihood tree) based on a 562 bp fragment of the mitochondrial gene *COI* from *Corbicula* spp. (modified from Pigneur *et al.*, 2011b). Bootstrap values for 1000 replications are indicated. Sperm type (mono- or biflagellate) is represented when known. Biflagellate sperm is indicative of androgenesis in *Corbicula* spp. Invasive lineages are indicated in bold. Origin and GenBank accession numbers of sequences are presented in Table 3. The cytonuclear mismatches (mitochondrial vs nuclear data) represented here are those documented in Lee *et al.* (2005), Pfenninger *et al.* (2002), Hedtko *et al.* (2008) and Pigneur *et al.* (2011b).

of androgenetic lineages (Lee *et al.*, 2005; Glaubrecht *et al.*, 2006; Hedtko *et al.*, 2008; Pigneur *et al.*, 2011b; Figure 3). Many of these authors agree, however, that mitochondrial phylogenies poorly resolve taxonomic relationships among freshwater *Corbicula* lineages and may even constitute a 'pitfall' by pooling divergent nuclear lineages together (Park and Kim, 2003; Lee *et al.*, 2005; Hedtko *et al.*, 2008; Pigneur *et al.*, 2011b). A combination of nuclear and mitochondrial data is therefore required to elucidate the relationships and evolutionary origin of androgenetic *Corbicula* lineages. Unfortunately, most nuclear markers analyzed in *Corbicula* have been either dominant or multi-copy (Park and Chung, 2003; Lee *et al.*, 2005; Hedtko *et al.*, 2008), and nuclear phylogenies based on single-copy genes are not phylogenetically congruent (Hedtko *et al.*, 2011). *Corbicula* lineages appear to have recently diverged (Glaubrecht *et al.*, 2006; Hedtko *et al.*, 2011), which may complicate resolution of trees based on DNA sequence data. Microsatellite markers have recently been developed by Pigneur *et al.* (2011a), and these may be useful in unravelling evolutionary relationships among *Corbicula*.

Recent analyses of nuclear markers do provide some information about evolutionary relationships across invasive species. The dominant American form A and European form R share exactly the same mitochondrial haplotype and nuclear genotype (Hedtko *et al.*, 2011; Pigneur *et al.*, 2011a). The American form B and the European form Rlc (from Rhône river) share the same mitochondrial lineage, but have distinctive morphology and belong to distinct nuclear genotypes (Hedtko and Hillis, 2011; Pigneur *et al.*, 2011a). One of these latter forms (B or Rlc) could have captured and become fixed for the mitochondrial DNA of the other form. In contrast, the South American form C and European form S present distinct morphotypes, but share both the same mitochondrial haplotype and nuclear DNA sequence (Hedtko and Hillis, 2011). This could suggest, among others, that for this latter *Corbicula* lineage, morphological distinctiveness may reflect phenotypic plasticity rather than distinct nuclear genotypes.

### POLYPLOIDY AND NUCLEAR CAPTURE

The most characteristic feature of asexuals is that they remain at the same ploidy level throughout their life cycle. Androgenetic *Corbicula* lineages range from diploidy to tetraploidy (Table 2), and in all cases maintain ploidy throughout life cycle via unreduced sperm. Although polyploids are relatively rare in bivalves, *Corbicula* is not the only genus that contains polyploids; polyploidy has also been found in asexuals of the marine gynogenetic genus *Lasaea* (Ó Foighil and Thiriou-Quévieux, 1991), several sexually reproducing species of the freshwater Sphaeriidae (Lee and Foighil, 2002) and in a few individuals of the sexual mussel *Mytilus trossulus* (González-Tizón *et al.*, 2000).

Both diploid and triploid (and probably tetraploid) androgenetic *Corbicula* produce unreduced spermatozoa (Komaru *et al.*, 1997). This suggests that diploid androgenetic individuals may evolve from a diploid ancestral population with normal meiosis (Qiu *et al.*, 2001). In androgenetic *Corbicula*, polyploidy could help maintain functional loci even though deleterious mutations may be accumulating (Lokki, 1976), assuming deleterious mutations are predominantly recessive. Polyploids with odd ploidy levels could be unable to produce viable gametes owing to difficulties in pairing more than two homologous chromosomes during meiosis. Polyploids lacking functional meiosis may thus be more likely to persist. In *Corbicula*, polyploidy could occur as a result of androgenesis: on one hand, unreduced sperm are produced, whereas on the other hand, incomplete polar body formation can occur when one or two maternal chromosome sets are not

expelled from the cytoplasm during androgenesis (Qiu *et al.*, 2001). Komaru *et al.* (2001) demonstrated that diploid androgenetic parents sometimes produce triploid embryos. In a few cases, eggs underwent the typical meiotic process and a 'female' pronucleus was also formed and fused with the unreduced male pronucleus. The offspring was therefore triploid. Incomplete polar body formation and formation of a female pronucleus has been observed in *C. leana* and *C. fluminea* (Komaru *et al.*, 2001, 2006). Alternatively, ploidy elevation could occur through retention of a polar body; the molecular mechanism in this case differs, but the result is the same: formation of a haploid female pronucleus, which can fuse with the male pronucleus, resulting in a triploid zygote. Furthermore, if the formation of the second polar body also fails, the zygote could be tetraploid.

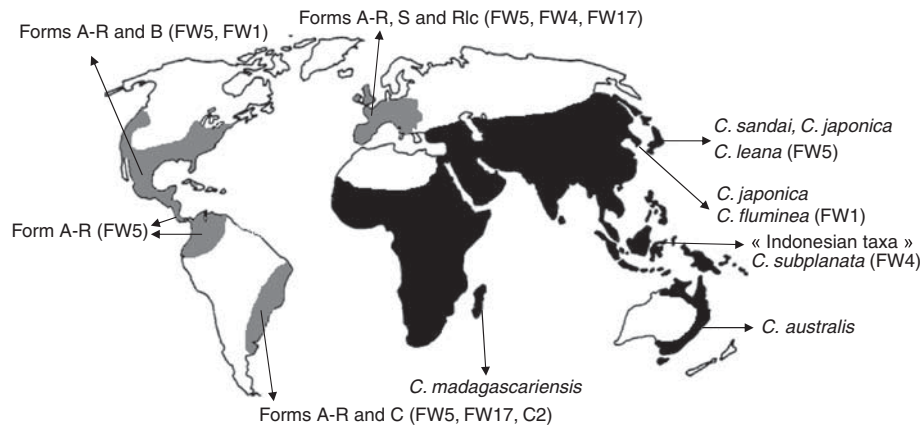
Nuclear capture occurs when part or all of the maternal nuclear chromosomes are retained. When this occurs between distinct evolutionary lineages, signs of such nuclear capture can be detected easily. Pfenninger *et al.* (2002) suggested evidence for possible nuclear hybridization between forms R and S in the River Rhine where both forms co-occur. In contrast, there was no evidence of current nuclear exchange between North American forms A and B based on ribosomal DNA markers (Lee *et al.*, 2005; Hedtko *et al.*, 2008), except in the Ohio River (Pigneur, unpublished data). The recent development of microsatellite markers for *Corbicula* offers the potential for in-depth examination of nuclear exchange at the population level.

### 'THE CLONE WARS': *CORBICULA* CLAMS AS SUCCESSFUL INVADERS

Whereas the androgenetic Tassili cypress is a rare, endangered species, androgenetic *Corbicula* are successful invaders worldwide (Figure 4). In freshwater ecosystems, *Corbicula fluminea sensu lato* (a designation that certainly contains several species; see for example, Hillis and Patton, 1982) has been reported as one of the most invasive species because of its wide geographic distribution and high invasive success (reviewed in Sousa *et al.*, 2008). This latter was mainly attributed to biological characteristics such as early maturation, high fecundity and excellent dispersal capacities through passive transportation of juveniles in water current or via aquatic birds. The native range of the genus *Corbicula* includes Asia, Australia, Africa and the Middle East (Araujo *et al.*, 1993; Figure 4). *Corbicula* clams were introduced into North America in the 1920s and rapidly spread throughout the continent (McMahon, 1982). They invaded Europe around 1980 (Mouthon, 1981) and are now widespread in most European countries. *Corbicula* clams are considered major invasive exotic species because of their impact on aquatic ecosystems (for example, Fuller and Imlay, 1976; Cohen *et al.*, 1984) and industrial cooling systems (Isom, 1986). Identical or closely related haplotypes of androgenetic lineages of *Corbicula* are found over large geographic distances both in native and invaded areas, whereas sexual lineages appear to be geographically restricted to their native range (von Rintelen and Glaubrecht, 2006; Pigneur *et al.*, 2011b; Table 3 and Figures 3 and 4).

Androgenetic reproduction might have played an important role in the invasive success of freshwater *Corbicula* clams (Pigneur *et al.*, 2011b). Androgenetic *Corbicula* are hermaphroditic (except the recent discovery of males in Japan; Houki *et al.*, 2011) and capable of self-fertilization. If a newly colonized niche is suitable, asexual lineages will not suffer an ecological disadvantage and are expected to thrive and spread: only one individual is sufficient to establish a new population, and each individual can have as many as 90 000 offspring in one breeding season (McMahon, 1999). If there is sufficient clonal diversity either due to colonization by diverse genotypes or due to new mutations, a successful clone with a broad tolerance could be





**Figure 4** Schematic map showing the current distribution of the main lineages of *Corbicula* spp. Native and invasive approximate ranges are indicated in black and gray, respectively (modified from McMahon, 1999; Lee *et al.*, 2005). Morphotypes are indicated and corresponding mitochondrial haplotypes (COI) are within parenthesis.

selected for and in the long term a general-purpose genotype may evolve (Van Doninck *et al.*, 2002).

Many invasive species exhibit increased genetic diversity owing to multiple introductions (for example, Kolbe *et al.*, 2004; Lavergne and Molofsky, 2007). However, several species are successful invaders despite a very low genetic diversity and this is known as the ‘genetic paradox’ of invasive species (Allendorf and Lundquist, 2003). Invasive *Corbicula* are characterized by a very low genetic diversity over large geographic distances on several continents (Kijviriya *et al.*, 1991; Siripattawan *et al.*, 2000; Lee *et al.*, 2005; Hedtke *et al.*, 2008; Pigneur *et al.*, 2011a, b). Within each morphologically distinct form of invasive *Corbicula*, there is virtually no genetic variation within and between populations. For example, in Europe, there are three mitochondrial lineages (R, S and Rlc); within one mitochondrial lineage, all tested individuals have exactly the same mitochondrial haplotype for two sequenced genes and the same nuclear genotype for 11 microsatellites (Pigneur *et al.*, 2011a, b). When individuals exhibit a mitochondrial/morphotype mismatch, the nuclear genotype is congruent with their morphology. In North American populations, each nuclear genotype also corresponds to a specific morphotype, and yet both mitochondrial lineages are found in each morphotype. Presumably, each clam harbors the same genotype as the ‘paternal’ lineage, which ‘parasitized’ the egg of the second lineage (Lee *et al.*, 2005; Hedtke *et al.*, 2008; Pigneur *et al.*, 2011b). In addition, across continents, populations of American form A and European form R appear identical at the mitochondrial and nuclear level (Hedtke *et al.*, 2011; Pigneur *et al.*, 2011a, b), indicating one widespread superclone (Pigneur *et al.*, 2011b; Figure 4). This lack of genetic variation could originate from a recent invasion by one or a few androgenetic *Corbicula* individuals of each form—the founder’s effect—or invasive populations could have gone through recent genetic bottlenecks. Indeed, the first study including mitochondrial and microsatellite markers of several native Asian populations revealed a higher genetic diversity in native populations both at the mitochondrial and nuclear level than in invading populations (Pigneur, unpublished data). Subsequently, invasive populations maintain this low genetic diversity because of asexual reproduction (androgenesis).

Phenotypic plasticity, which is known to be high in molluscs, might have favored *Corbicula*’s adaptation to new environments and their invasion, as in many other invasive species (Dybdhal and Kane, 2005; Geng *et al.*, 2007; Poulin *et al.*, 2007). The invasive clonal lineages of

*Corbicula* in Europe and the Americas could be general-purpose genotypes associated with high levels of plasticity and broad environmental tolerance (Vrijenhoek and Parker, 2009; Le Roux *et al.*, 2007). Alternatively, they could be opportunistic specialists (similar to the invasive aquatic snail *Potamopyrgus antipodarum*; Drown *et al.*, 2010; see also Van Doninck *et al.*, 2003). Indeed, form C from South America and form S from Europe are morphologically, but not genetically, distinct; the same is true of form A from North America and form R from Europe, although their morphology seems more similar (Hedtke *et al.*, 2011; Pigneur *et al.*, 2011a).

Nevertheless, a certain degree of genetic polymorphism may be found in *Corbicula* when there is a combination of a nuclear genome of one lineage with a new mitochondrial genome of a different lineage as a result of ‘egg parasitism’ between lineages. This could provide a higher advantage to mixed cytonuclear lineages if the interactions between cytoplasmic genes from one lineage and nuclear genes from another are beneficial, or these interactions could be deleterious, but the mixed genotype spreads due to genetic drift. Thorsness and Weber (1996) emphasized the importance of studying the effects of interactions between mitochondria and nuclei on rates of molecular evolution; their study suggests that in yeasts, the rate of escape of gene-sized fragments of mitochondrial DNA towards the nucleus is more or less similar to the rate of spontaneous mutations of nuclear genes. Further genetic diversity within a lineage could be generated by rare nuclear genetic capture between divergent lineages (Hedtke and Hillis, 2011). Ultimately, divergence between alleles within a lineage could accumulate over time (the ‘Meselson effect’; Judson and Normark, 1996), although there is no evidence that different lineages of *Corbicula* have yet accumulated independent mutations between alleles (Hedtke *et al.*, 2011).

#### A THIN BORDER BETWEEN SEXUALITY AND ASEQUALITY

Are androgenetic *Corbicula* clams definitely asexual? Scali (2009) (in Schön *et al.*, 2009) stated that androgenetic reproduction ‘resembles sexual reproduction with most of its genetic traits’. Mitochondria seem relatively easy to exchange between lineages; this has occurred in both Europe and America. Although the lack of genetic diversity within invasive populations suggests that true hybridization is rare, there is evidence for nuclear hybridization between diverse *Corbicula* lineages (Pfenninger *et al.*, 2002; Hedtke *et al.*, 2011). While nuclear DNA is not regularly captured, mitochondrial DNA capture appears to be

common. Is this sex? We would argue that in nature, reproduction is not a discrete trait with only two values (asexual vs sexual). Instead, there is a continuum between parthenogenesis without meiosis or recombination on one end and sexual reproduction on the other. This continuum harbors a fascinating diversity of reproductive modes with behaviour retained from sexual ancestors, including meiosis, recombination, gametic interactions and even physical and chemical sexual stimulations. From a genic perspective, androgenetic *Corbicula* regularly pass on nuclear chromosomes from only one parent, the father, and from that perspective are asexual. They rarely do incorporate DNA—nuclear or mitochondrial—from other lineages, but this does not appear to be common and this is a feature shared by other 'asexual' organisms. From a life-cycle perspective, androgenetic *Corbicula* do not normally change ploidy level between generations, and are thus asexual. That said, ploidy can change when a maternal haploid chromosome set is added to the diploid (or triploid) paternal chromosome set. Thus, in our continuum, *Corbicula* are more asexual than sexual; they are interesting in part because they are not paternal versions of true parthenogens, and because they have this capacity for incorporating novel genetic material and even entire chromosomes: the capacity for rare sex.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

We are grateful to Dr D Aldridge for samples from Ohio River and to the Editor and two anonymous reviewers for their valuable suggestions and comments.

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