Spermatozoa production by triploid males in the New Zealand freshwater snail *Potamopyrgus antipodarum*

DEANNA M. SOPER1*, MAURINE NEIMAN2, OLEKSANDR P. SAVYTSKYY1, MIRIAM E. ZOLAN1 and CURT M. LIVELY1

1Department of Biology, Indiana University, Bloomington, IN, USA
2Department of Biology, University of Iowa, Iowa City, IA, USA

Received 13 February 2013; revised 3 March 2013; accepted for publication 5 March 2013

Asexual lineages derived from dioecious taxa are typically assumed to be all female. Even so, asexual females from a variety of animal taxa occasionally produce males. The existence of these males sets the stage for potential gene flow across asexual lineages as well as between sexual and asexual lineages. A recent study showed that asexual triploid female *Potamopyrgus antipodarum*, a New Zealand freshwater snail often used as a model to study sexual reproduction, occasionally produce triploid male offspring. Here, we show that these triploid male *P. antipodarum* (1) have testes that produce morphologically normal sperm, (2) make larger sperm cells that contain more nuclear DNA than the sperm produced by diploid sexual males, and (3) produce sperm that range in DNA content from haploid to diploid, and are often aneuploid. Analysis of meiotic chromosomes of triploid males showed that aberrant pairing during prophase I probably accounts for the high variation in DNA content among sperm. These results indicate that triploid male *P. antipodarum* produce sperm, but the extent to which these sperm are able to fertilize female ova remains unclear. Our results also suggest that the general assumption of sterility in triploid males should be more closely examined in other species in which such males are occasionally produced. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, 110, 227–234.

ADDITIONAL KEYWORDS: gametogenesis – ploidy evolution – polyploidy – reproduction – sperm – triploid fertility.

INTRODUCTION

The assumption that asexual lineages produce only daughters is known to be violated in a variety of taxa (Hebert et al., 1989; Browne, 1992; Butlin, Schon & Martens, 1998; Smith, Kamiya & Horne, 2006; Lunt, 2008). Male production by asexual females (or maintenance of male function in hermaphrodites) could be of evolutionary importance for at least four reasons: (1) it could reduce the two-fold cost of males expected to be experienced by sexuals competing with coexisting asexuals (Neiman et al., 2012), (2) it could reduce the fitness of male-producing asexual lineages compared with competing asexual lineages that produce only females (Innes, Fox & Winsor, 2000), (3) it raises the potential for gene flow among asexual lineages and between sexual and asexual lineages (Harlan & deWet, 1975; Husband, 2004), and (4) it may assist in the maintenance of coexistence between asexual and sexual lineages (Mogie, 2011).

Asexual animal lineages are very often polyploid (Suomalainen, Saura & Lokki, 1987; Otto & Whitton, 2000), and it is generally assumed that abnormalities in testicular tissue, spermatogenesis, genetic content, and/or sperm morphology in males produced by asexual polyploid females will render these males sterile (Kawamura et al., 1999; Carrell et al., 2004; Mable, 2004; Hamaguchi & Sakaizumi, 2005; Rives et al., 2005). One example of probable sterility in triploid males is provided by the loach *Misgurnus anguillicaudatus*; the majority of sperm produced by these males lack flagella (Oshima et al., 2005). Nevertheless, fertile triploid males do occur in a variety of invertebrate and vertebrate animal taxa such as the planarian *Schmidtea polychroa*, the tench *Tinca tinca*.
(L.), and the Iberian minnow Squalius alburnoides (Linhart et al., 2006; Sousa-Santos, Collares Pereira & Almada, 2007; D’Souza et al., 2008; Hulak et al., 2010).

Potamopyrgus antipodarum, a New Zealand freshwater snail, is characterized by frequent coexistence between diploid sexual individuals (male and female) and polyploid asexual individuals (usually females) (Lively, 1987; Dybdahl & Lively, 1995). Asexual assemblages of P. antipodarum are genetically diverse (Fox et al., 1996; Jokela et al., 2003) and feature extensive within- and across-population ploidy-level variation (Neiman et al., 2011). A recent study documented the existence of polyploid P. antipodarum with an external male genital structure at low (~5%) frequency within lake populations (Neiman et al., 2011; see also Lively & Jokela, 2002) and a follow-up experiment demonstrated that these males are produced by asexual polyploid females (Neiman et al., 2012).

We used triploid P. antipodarum that bear external male genital structures to take critical steps towards evaluating the conventional assumption that triploid males produced by asexual triploid females are sterile. There are at least five necessary components that must be in place if triploid males are to make a genetic contribution to future generations. First, triploid males must contain reproductive tissues that are able to produce sperm. Second, copulation must result in transfer of sperm to the female reproductive tract. Third, sperm must fertilize ova. Fourth, the embryos must develop into viable offspring. Fifth, the offspring must successfully mature and reproduce. We focused on step one: determining whether triploid males have testes, and if they do, whether they produce sperm. We also compared the size, morphology, and DNA content of sperm produced by triploid males with sperm produced by sexual diploid male P. antipodarum and used cytological techniques to examine chromosomal pairing during spermatogenesis in diploid and triploid males.

MATERIAL AND METHODS

IDENTIFICATION OF TESTES AND SEMINAL VESICLE TISSUE

An external genital structure, located just posterior to the head on the right side, was used to distinguish male from female P. antipodarum (e.g. Nelson & Neiman, 2011). Diploid male P. antipodarum are known to be sexual (Phillips & Lambert, 1989; Wallace, 1992), and we thus used males from an established sexual, diploid laboratory stock to locate and identify testes tissue and sperm. Snails maintained in the laboratory are kept at a constant 16–17 °C in square containers that hold 2 L of water with a 12-h light cycle and are fed a solution of dried Spirulina algae three times per week. The exact location of the testes and seminal vesicle were identified via dissections, and tissue verification was conducted by use of light microscopy (Fig. 1). Dissections were conducted in saline solution so that, if present, sperm were kept alive for observations.

We compared these diploid male P. antipodarum with the 10 putatively triploid males found within a sample of ~1000 snails collected from deep regions (~6 m in depth) of Lake Alexandrina, New Zealand, during January 2009. We used males from this sample because previous studies indicated that males...
collected from deep-water habitats in Lake Alexandrina are almost always triploid (Lively, 1987; Dybdahl & Lively, 1995; Lively & Jokela, 2002; Neiman et al., 2011). These field-collected *P. antipodarum* were transported to Indiana University and maintained under standard laboratory conditions for *P. antipodarum* (described above) for approximately 6 months and then dissected to compare the internal morphology with diploid males (Fig. 1). We also used flow cytometry (methods given below) to confirm the ploidy level of these putatively triploid males.

**Sperm collection and measurement**

We used sperm extracted from 35 diploid and six triploid males (obtained from the same sources as above) to determine whether there were morphological and/or size differences between sperm produced by males of the different ploidy levels. We began by dissecting out the seminal vesicle and placing it in freshwater (which killed the sperm on contact), so that pictures and measurements of non-motile sperm could be taken. Sperm were extracted from the seminal vesicle with a thin needle, placed on a new glass slide, and dispersed by sliding a coverslip over the sperm-containing droplet of water. Five sperm from each individual were located using brightfield light microscopy on a Nikon E800 microscope, and an image of each sperm was taken and measured using the Metamorph imaging system (Molecular Devices, Sunnyvale, CA, USA). The head and tail of each sperm was measured length-wise from tip to tip.

**Flow cytometry analysis of sperm**

We used flow cytometric analysis of sperm DNA content to determine the ploidy level of sperm produced by triploid males. Because only a small portion of sperm was used for the sperm-size measurements detailed above, we were able to flash-freeze and store the rest of the sperm and the seminal vesicle from the males used for the sperm-size study for flow cytometry analysis of nuclear DNA content. We prepared samples for flow cytometry by thawing them, adding 750 μL of cold DMSO, and then briefly grinding with a motorized grinder and pestle. We then used a mix of propidium iodide, spermine, and detergent stock solution to stain nuclear DNA (Osnas & Lively, 2006). Stained samples were filtered through a Partec Celltric 50-μm mesh filter and analysed with a FACS Calibur (BD Biosciences, Franklin Lakes, NJ, USA) flow cytometer at Indiana University. Somatic tissue was used for flow cytometry (methods as above) to determine whether there were morphological and/or size differences between sperm produced by triploid and diploid males and to compare those results with triploid and diploid *P. antipodarum* somatic tissue.

A random subset of samples was shipped to Amnis Corporation (Seattle, WA, USA) for analysis on an ImageStreamX Cytometer, which takes an image of each cell being examined. This method allowed us to verify that the cells being analysed were from a population of cells that were sperm. Diploid and triploid males were dissected as described above. Seminal vesicles were frozen in liquid nitrogen and shipped overnight on dry ice to Amnis. Amnis conducted cell preparation, which included DNA staining using DAPI.

Because samples were run on two different flow cytometers (at Amnis and Indiana University), we divided the mean fluorescence of each sample by the mean fluorescence of the haploid standard run on the same cytometer on the same day to calculate a standardized fluorescence value for each sample. We used these standardized values for all subsequent statistical analyses.

**Cytological analysis of testes tissue**

Diploid sexual males were obtained from the laboratory lineage described above. Triploid males were born and raised in the lab, but were descended from triploid females collected from deep regions of Lake Alexandrina (~6 m in depth) in New Zealand in 2009. Testes and seminal vesicle tissues were dissected, and head tissue was used to confirm triploid chromosomal composition via flow cytometry (methods as above). We then used electron microscopy to compare the spermatogenesis process of seven diploid and seven triploid males. First, we immediately submerged the testes tissue in 100 μL of 0.9% sodium citrate in a 1.5-mL microcentrifuge tube and gently shook the tubes for 30 min. Next, we homogenized the tissue with a plastic pestle attached to a handheld drill. After 5 min of drilling, we spun the homogenate for 10 min at 2000 r.p.m. with a desktop microcentrifuge. We carefully removed the supernatant with a pipette and then resuspended the pellet with 50 μL of 4% paraformaldehyde. Spreading and staining techniques were the same as per Pukkila and colleagues (Pukkila & Lu, 1985; Pukkila, Skrzynia & Lu, 1992), except that spreading dishes and slides were coated with 0.9% plastic solution. Spreading dishes were allowed to dry for at least 24 h before use, but slides were used within 2–4 h after coating, which facilitated subsequent plastic removal. We also departed from Pukkila et al. (1992) for the wash step in dipping slides in a Kodak Photo-Flo 200 solution only 3–5
times to minimize the amount of spread lost and in omitting the water-washing step. Images from prepared slides were taken using a JEOL-1010 electron microscope. We then used these images to analyse synaptonemal complex (SC) formation in cells undergoing gametogenesis. Examining the SC and association of homologous chromosome pairs also allowed for verification of ploidy of the individual.

STATISTICAL ANALYSIS

The head and tail for each of five sperm from each individual were measured and recorded. Differences in sperm length (head and tail) between ploidy groups were analysed by generating a mean sperm head and tail length for each individual and then using an independent-samples t-test to compare the means of diploid and triploid males.

Mean nuclear DNA content of ploidy groups was obtained by calculating mean fluorescence for each individual through use of flow cytometry. We used an independent-samples t-test to compare these individual means between diploid and triploid males. All statistical analyses were performed with SPSS version 19 (IBM).

RESULTS

COMPARISON OF TESTES TISSUE AND SPERM FROM DIPLOID AND TRIPLOID MALES

The testes of male P. antipodarum are internal, dark orange in colour, and reside on the dorsal surface of the soft body tissue. The seminal vesicle, a white tube structure, exits the anterior portion of the testes, which is densely packed with sperm. Triploid and diploid male testes were visually indistinguishable (Fig. 1A, B) and both exhibited mobile sperm. While the morphology of the sperm produced by diploid and triploid males did not visibly differ in structure (Fig. 2A, B), quantitative comparisons between triploid and diploid sperm revealed that triploid sperm heads and tails were ~19.2 and 14.2% larger, respectively, than their diploid counterparts. Independent t-tests indicated that both size differences were significant (Heads: diploid mean ± SE = 4.7 ± 0.33 μm; triploid mean = 5.8 ± 0.15 μm; t = −10.9, P = 0.001, d.f. = 39; Tails: diploid mean = 95.9 ± 0.83 μm; triploid mean = 111.9 ± 1.31 μm; t = −7.66, P < 0.0001, d.f. = 39).

FLOW CYTOMETRY COMPARING DIPLOID AND TRIPLOID MALES

We used flow cytometry to analyse the nuclear DNA content of sperm produced by eight diploid males and eight triploid males. We assigned the ploidy of sperm as haploid, diploid, or aneuploid; the last designation was applied to sperm with DNA content between that of known haploid and diploid standards. The analysis of sperm produced by diploid males yielded a narrow peak corresponding to haploid nuclear DNA content (Fig. 3). In contrast, analysis of sperm produced by triploid males yielded a relatively broad distribution of DNA content that ranged from haploid to diploid (Fig. 3). We also found that the nuclear DNA content of sperm from diploids (N = 8; mean = 0.483; SE = 0.025) was significantly lower than that of sperm from triploid males (N = 8; mean = 0.684; SE = 0.018) (t = −6.45; P = 0.001, d.f. = 14).

Figure 2. No major morphological differences exist between sperm extracted from diploid and triploid males: A, representative sperm from diploid male; B, representative sperm from triploid male. Scale bars = 30 μm.

Figure 3. Dotted line indicates typical flow cytometry fluorescence from the sperm of a triploid male. Solid line indicates typical flow cytometry fluorescence from the sperm of a diploid male.
CYTOLOGICAL ANALYSIS OF TESTES TISSUE

Electron microscopic analysis of surface spreads of meiotic chromosomes showed consistent SC formation in diploid males (Fig. 4). This type of pairing is indicative of balanced chromosomal segregation that leads to haploid gametes (Zickler & Kleckner, 1999; Egozcue et al., 2000; Oliver-Bonet et al., 2004). Light and electron microscopy analysis through chromosomal counting confirmed that diploid individuals contain 17 pairs of chromosomes, as reported by Wallace (1992). Triploid males had mixes of bivalents and univalents as well as aberrant figures in which ‘partner switching’ occurs (this was observed in 11 of 12 spreads). In partner switching, an individual chromosome synapses with portions of two other chromosomes (Fig. 5, arrow 2). Abnormal synapses most likely lead to unbalanced chromosomal numbers in the resulting gametes (Jackson, 1976; Bretagnolle & Thompson, 1995).

DISCUSSION

We found that triploid male P. antipodarum produce sperm that are morphologically similar to sperm produced by diploid males, but triploids have significantly larger heads and tails. Cytometric and cytological results suggest that sperm produced by triploid males often exhibit aneuploidy, and indicate that although triploid males undergo meiosis, the chromosomal pairing is often aberrant. These latter results are of particular interest because unreduced and/or abnormal chromosomal number in gametes is suspected to be a common source of polyploidy (Bretagnolle & Thompson, 1995; Ramsey & Schemske, 1998; Koutecký et al., 2011). One relevant example comes from Arabidopsis thaliana, in which restitution of either the first or the second meiotic division can result in the production of offspring with higher levels of ploidy than their parents (Bretagnolle & Thompson, 1995; Köhler, Mittelsten Scheid & Erilova, 2010). A few documented cases of ploidy elevation caused by gametes with an abnormal chromosome number exist in animals. One such example comes from artificially generated triploid and natural tetraploid loaches (Misgurnus anguillicaudatus). Here, eggs produced by tetraploid females are fertilized with sperm (mostly aneuploid) produced by triploid males, resulting in offspring with higher levels of ploidy than their parents (Zhang & Arai, 1999). This example indicates that even in animals, aneuploid sperm should not be discounted.

Sperm competition can influence the evolution of sperm morphology, with the prediction that variation in sperm morphology will be negatively correlated with the intensity of sperm competition (Lifjeld et al., 2013). While the relationship between sperm morphology and fertilization success remains unclear (Gomendio & Roldan, 1991; Briskie & Montgomerie, 1992; Birkhead & Møller, 1998; LaMunyon &
Ward, 1998; Miller & Pitnick, 2002; Snook, 2005; Garcia-Gonzalez & Simmons, 2007; Birkhead, Hosken & Pitnick, 2009), there is evidence from at least one other snail species that larger sperm may outcompete smaller sperm (Oppliger et al., 2003). It is thus possible that the larger sperm produced by triploid male P. antipodarum may at times outcompete the smaller sperm of diploid males in the many populations in which triploid and diploid male P. antipodarum coexist (Neiman et al., 2011).

The frequent aneuploidy of sperm produced by triploid males may mean that the fertilized eggs are less likely to develop into viable offspring than eggs fertilized by a normal haploid sperm. However, triploid males are found at about ~5% frequency in otherwise all-female and all-polyploid populations (Lively & Jokela, 2002; Neiman et al., 2011). Because diploid males are absent or extremely rare in these lakes, sperm produced by triploid males represent the only possibility of fertilization success within these populations. However, because sperm morphology may be affected by environmental conditions (Gage & Cook, 1994; Hellriegel & Blanckenhorn, 2002; Amitin & Pitnick, 2006; Birkhead et al., 2009), it is important to note that we cannot formally exclude the possibility that the different rearing environments of field-collected triploid males and the laboratory-reared diploid males could have differentially influenced sperm morphology. Future studies on laboratory-raised triploid males are needed to confirm that the observed differences in sperm head and tail size between diploid and triploid males hold up regardless of rearing environment.

In conclusion, we have demonstrated that triploid male P. antipodarum have testes and sperm, setting the stage for the possibility that these males can be a source of gene flow among asexual lineages and between sexual and asexual P. antipodarum. More broadly, these results show that polyploid males produced by asexual polyploid females may not be sterile. In addition, we have shown clear differences in the size of sperm produced by laboratory-reared diploid males and field-collected triploid males. We have also contributed towards understanding the cause of aneuploidy observed in the sperm of triploid males by documenting partner switching during meiotic chromosome synapsis. A complete understanding of the extent to which these males can provide sperm of diploid origin is underway. Future studies on laboratory-raised triploid males are needed to confirm that the observed differences in sperm head and tail size between diploid and triploid males hold up regardless of rearing environment.

ACKNOWLEDGEMENTS

We thank Jim Powers and the Indiana University Light Microscopy Center, Dr. F. Rudolf Turner for taking the electron micrographs, Dai Tsuji for assisting with developing cytological protocols, and Stephanie Dickenson at the Indiana University Statistical Counseling Center. We also thank Raymond Kong and Jennifer Darnell at Amnis Corporation for assistance with visual confirmation of cellular material during flow cytometry. Research in the Zoltn lab was supported by grant GM43930 from the National Institutes of Health and by a supplement to this grant from the American Recovery & Reinvestment Act of 2009. This research was supported in part by the Indiana METACyt Initiative of Indiana University, funded in part through a major grant from the Lilly Endowment, Inc. We also thank three anonymous reviewers for their helpful comments.

REFERENCES

Amitin EG, Pitnick S. 2006. Influence of developmental environment on male- and female-mediated sperm precedence in Drosophila melanogaster. Journal of Evolutionary Biology 20: 381–391.

Birkhead TR, Hosken DJ, Pitnick S. 2009. Sperm biology: an evolutionary perspective. New York: Academic Press.

Birkhead TR, Møller AP. 1998. Sperm competition and sexual selection. New York: Academic Press.

Bretagnolle F, Thompson JD. 1995. Tansley review No. 78. Gametes with the stomatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. New Phytologist 129: 1–22.

Briskie JV, Montgomerie R. 1992. Sperm size and sperm competition in birds. Proceedings of the Royal Society of London. Series B: Biological Sciences 247: 89–95.

Browne RA. 1992. Population genetics and ecology of Artemia: insights into parthenogenetic reproduction. Trends in Ecology & Evolution 7: 232–237.

Butlin R, Schön I, Martens K. 1998. Asexual reproduction in nonmarine ostracods. Heredity 81: 473–480.

Carrell DT, Emery BR, Wilcox AL, Campbell B, Erickson L, Hatassaka HH, Jones KP, Peterson CM. 2004. Sperm chromosome aneuploidy as related to male factor infertility and some ultrastructure defects. Systems Biology in Reproductive Medicine 50: 181–185.

D’Souza TG, Bellenhaus V, Wesselmann R, Michiels NK. 2008. Sperm length and quality in sperm-dependent parthenogens. Biological Journal of the Linnean Society 93: 81–87.

Dybdahl MF, Lively CM. 1995. Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail (Potamopyrgus antipodarum). Journal of Evolutionary Biology 8: 385–398.

Egozcue S, Blanco J, Vendrell JM, Garcia F, Veiga A, Aran B, Barri PN, Vidal F, Egozcue J. 2000. Human
male infertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. Human Reproduction Update 6: 93–105.

Fox JA, Dybdahl MF, Jokela J, Lively CM. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (Potamopyrgus Antipodarum). Evolution 50: 1541–1548.

Gage MJG, Cook PA. 1994. Sperm size or numbers? Effects of nutritional stress upon eyeprene and apyrene sperm production strategies in the moth Plodia interpunctella (Lepidoptera: Pyralidae). Functional Ecology 8: 594–599.

Garcia-González F, Simmons LW. 2007. Shorter sperm confer higher competitive fertilization success. Evolution 61: 816–824.

Gomendio M, Roldan ERS. 1991. Sperm competition influences sperm size in mammals. Proceedings of the Royal Society of London. Series B: Biological Sciences 243: 181–185.

Hamaguchi S, Sakaizumi M. 2005. Sexually differentiated mechanisms of sterility in interspecific hybrids between Oryzias latipes and O. curvinitos. Journal of Experimental Zoology 293: 323–329.

Harlan JR, deWet JMJ. 1975. On O. Winge and a prayer: the origins of polyplody. The Botanical Review 41: 361–390.

Hebert PDN, Beaton MJ, Schwartz SS, Stanton DJ. 1989. Polyphyletic origins of asexuality in Daphnia pulex. I. breeding-system variation and levels of clonal diversity. Evolution 43: 1004–1015.

Hellriegel B, Blanckenhorn WU. 2002. Environmental influences on the gametic investment of yellow dung fly males. Evolutionary Ecology 16: 505–522.

Hulak M, Kaspar V, Psenicka M, Gela D, Li P, Linhart O. 2010. Does triploidization produce functional sterility of triploid males of tench Tinca tinca (L.)? Reviews in Fish Biology and Fisheries 20: 307–315.

Husband BC. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. Biological Journal of the Linnean Society 82: 537–546.

Innes DJ, Fox CJ, Winsor GL. 2000. Avoiding the cost of males in obligately asexual Daphnia pulex (Leydig). Proceedings of the Royal Society of London. Series B: Biological Sciences 267: 991–997.

Jackson RC. 1976. Evolution and systematic significance of polyploidy. Annual Review of Ecology and Systematics 7: 209–234.

Jokela J, Lively CM, Dybdahl MF, Fox JA. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. Biological Journal of the Linnean Society 79: 165–181.

Kawamura K, Ueda T, Aoki K, Hosoya K. 1999. Spermatozoa in triploids of the rosy bitterling Rhodeus ocellatus ocellatus. Journal of Fish Biology 55: 420–432.

Köhler C, Mittelsten Scheid O, Erilova A. 2010. The impact of the triploid block on the origin and evolution of polyploid plants. Trends in Genetics 26: 142–148.

Koutecký P, Badurová T, Stech M, Košnar JAN, Karásek J. 2011. Hybridization between diploid Centaurea pseudophrygia and tetraploid C. jacea (Asteraceae): the role of mixed pollination, unreduced gametes, and mentor effects. Biological Journal of the Linnean Society 104: 93–106.

LaMunyon CW, Ward S. 1998. Larger sperm outcompete smaller sperm in the nematode Caenorhabditis elegans. Proceedings of the Royal Society of London. Series B: Biological Sciences 265: 1997–2002.

Lifjeld JT, Hoenen A, Johannessen LE, Laskemoen T, Lopes RJ, Rodrigues P, Rowe M. 2013. The Azores bullfinch (Pyrrhula murina) has the same unusual and size-variable sperm morphology as the Eurasian bullfinch (Pyrrhula pyrrhula). Biological Journal of the Linnean Society 108: 677–687.

Linhart O, Rodina M, Flajshans M, Mavrodiev N, Nebsarova J, Gela D, Kocour M. 2006. Studies on sperm of diploid and triploid tench, Tinca tinca (L.). Aquaculture International 14: 9–25.

Lively C, Jokela J. 2002. Temporal and spatial distributions of parasites and sex in a freshwater snail. Evolutionary Ecology Research 4: 219–226.

Lively CM. 1987. Evidence from a New-Zealand snail for the maintenance of sex by parasitism. Nature 328: 519–521.

Lunt DH. 2008. Genetic tests of ancient asexuality in root knot nematodes reveal recent hybrid origins. BMC Evolutionary Biology 8: 194.

Mable BK. 2004. ‘Why polyploidy is rarer in animals than in plants’: myths and mechanisms. Biological Journal of the Linnean Society 82: 453–466.

Miller GT, Pitnick S. 2002. Sperm-female coevolution in Drosophila. Science 298: 1230–1233.

Mogie M. 2011. Pollen profile, spatial structure, and access to sex in asexual hermaphrodites. Biological Journal of the Linnean Society 103: 954–966.

Neiman M, Larkin K, Thompson AR, Wilton P. 2012. Male offspring production by asexual Potamopyrgus antipodarum, a New Zealand snail. Heredity 109: 57–62.

Neiman M, Paczesniak D, Soper DM, Baldwin AT, Hehman G. 2011. Wide variation in ploidy level and genome size in a new zealand freshwater snail with coexisting sexual and asexual lineages. Evolution 65: 3201–3216.

Nelson AE, Neiman M. 2011. Persistent copulation in asexual female Potamopyrgus antipodarum: evidence for male control with size-based preferences. International Journal of Evolutionary Biology 2011: 439046.

Oliver-Bonet M, Navarro J, Codina-Pascual M, Abad C, Guitart M, Egozcue J, Benet J. 2004. From spermatocytes to sperm: meiotic behaviour of human male reciprocal translocations. Human Reproduction 19: 2515–2522.

Oppliger A, Nacicri-Graven Y, Ribi G, Hosken DJ. 2003. Sperm length influences fertilization success during sperm competition in the snail Vitrupar asater. Molecular Ecology 12: 485–492.

Oshima K, Morishima K, Yamana E, Araki K. 2005. Reproductive capacity of triploid loaches obtained from Hokkaido Island, Japan. Ichthyological Research 52: 1–8.

Osnas EE, Lively CM. 2006. Host ploidy, parasitism and
immune defence in a coevolutionary snail–trematode system. *Journal of Evolutionary Biology* **19**: 42–48.

**Otto SP, Whitton J. 2000.** Polyploid incidence and evolution. *Annual Review of Genetics* **34**: 401–437.

**Phillips NR, Lambert DM. 1989.** Genetics of *Potamopyrgus antipodarum* (Gastropoda: Prosobranchia): evidence for reproductive modes. *New Zealand Journal of Zoology* **16**: 435–445.

**Pukkila PJ, Lu BC. 1985.** Silver staining of meiotic chromosomes in the fungus, *Coprinus cinereus*. *Chromosoma* **91**: 108–112.

**Pukkila PJ, Skrzynia C, Lu BC. 1992.** The Rad3 1 mutant is defective in axial core assembly and homologous chromosome pairing during meiosis in the basidiomycete *Coprinus cinereus*. *Developmental Genetics* **13**: 403–410.

**Ramsey J, Schemske DW. 1998.** Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29**: 467–501.

**Rives N, Mousset-Simeon N, Mazurier S, Mace B. 2005.** Primary flagellar abnormality is associated with an increased rate of spermatozoa aneuploidy. *Journal of Andrology* **26**: 61–69.

**Smith RJ, Kamiya T, Horne DJ. 2006.** Living males of the ‘ancient asexual’ Darwinulidae (Ostracoda: Crustacea). *Proceedings of the Royal Society B: Biological Sciences* **273**: 1569–1578.

**Snook RR. 2005.** Sperm in competition: not playing by the numbers. *Trends in Ecology & Evolution* **20**: 46–53.

**Sousa-Santos C, Collares Pereira MJ, Almada V. 2007.** Fertile triploid males – an uncommon case among hybrid vertebrates. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **307**: 220–225.

**Suomalainen E, Saura A, Lokki J. 1987.** Polyploidy in association with parthenogenesis. Boca Raton, FL: CRC Press, Inc.

**Wallace C. 1992.** Parthenogenesis, sex and chromosomes in *Potamopyrgus*. *Journal of Molluscan Studies* **58**: 93–107.

**Zhang Q, Arai K. 1999.** Aberrant meioses and viable aneuploid progeny of induced triploid loach (*Misgurnus anguillicaudatus*) when crossed to natural tetraploids. *Aquaculture* **175**: 63–76.

**Zickler D, Kleckner N. 1999.** Meiotic chromosomes: integrating structure and function. *Annual Review of Genetics* **33**: 609–754.