JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12403

REVIEW

Inbreeding depression in male gametic performance

S. LOSDAT*, S.-M. CHANG† & J. M. REID*

*Institute of Biological and Environmental Sciences, School of Biological Sciences, University of Aberdeen, Aberdeen, UK
†Plant Biology Department, University of Georgia, Athens, GA, USA

Keywords:

gamete competition;
inbreeding depression;
inbreeding load;
male reproductive success;
meta-analysis;
pollen performance;
pollination;
post-copulatory sexual selection;
sperm performance.

Abstract

One key objective in evolutionary ecology is to understand the magnitude of inbreeding depression expressed across sex-specific components of fitness. One major component of male fitness is fertilization success, which depends on male gametic performance (sperm and pollen performance in animals and plants, respectively). Inbreeding depression in male gametic performance could create sex-specific inbreeding depression in fitness, increase the benefit of inbreeding avoidance and reduce the efficacy of artificial insemination and pollination. However, there has been no assessment of the degree to which inbreeding generally depresses male gametic performance and hence post-copulatory or post-pollination fertilization success. Because inbreeding depression is understood to be a property of diploid entities, it is not clear what degree of inbreeding depression in haploid gametic performance should be expected. Here, we first summarize how inbreeding depression in male gametic performance could potentially arise through gene expression in associated diploid cells and/or reduced genetic diversity among haploid gametes. We then review published studies that estimate the magnitude of inbreeding depression in traits measuring components of sperm or pollen quantity, quality and competitiveness. Across 51 published studies covering 183 study traits, the grand mean inbreeding load was approximately one haploid lethal equivalent, suggesting that inbreeding depresses male gametic performance across diverse systems and traits. However, there was an almost complete lack of explicit estimates from wild populations. Future studies should quantify inbreeding depression in systematic sets of gametic traits under naturally competitive and noncompetitive conditions and quantify the degree to which gamete phenotypes and performance reflect haploid vs. diploid gene expression.

Introduction

Inbreeding, defined as reproduction among relatives, often decreases mean phenotypic trait values and fitness in offspring, a phenomenon known as inbreeding depression (Charlesworth & Charlesworth, 1987; Lynch & Walsh, 1998). There is ample evidence of inbreeding depression in individual reproductive success across wild, captive and experimental populations of plant and animal species (Crnokrak & Roff, 1999; Hedrick & Kalinowski, 2000; Meagher *et al.*, 2000; Keller & Waller, 2002). As a consequence, inbreeding depression has become a central paradigm in evolutionary ecology and conservation biology, particularly because it is expected to influence mating system evolution (Lande & Schemske, 1985; Schemske & Lande, 1985; Tregenza & Wedell, 2000; Pizzari *et al.*, 2004; Charlesworth, 2006) and reduce population growth rate and persistence (Charlesworth & Charlesworth, 1987; Saccheri *et al.*, 1998; Crnokrak & Roff, 1999; Hedrick & Kalinowski, 2000). However, the evolutionary and conservation consequences of inbreeding depression are expected to

Correspondence: Sylvain Losdat, Institute of Biological and Environmental Sciences, School of Biological Sciences, Zoology Building, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK. Tel.: +44 1224 274224; fax: +44 1224 272396;
e-mails: s.losdat@abdn.ac.uk and sylvain_losdat@yahoo.fr

depend on the degree to which it is expressed in males vs. females and across different life-history stages (Husband & Schemske, 1996; Szulkin *et al.*, 2007; Whitlock & Agrawal, 2009; Brekke *et al.*, 2010).

Male reproductive success can be an extremely variable fitness component within populations, creating substantial opportunities for selection and inbreeding depression (Clutton-Brock, 1988; Birkhead & Møller, 1998; Waller *et al.*, 2008). However, the mechanisms underlying inbreeding depression in male reproductive success, and the components of selection involved, remain unresolved. Inbreeding depression can be partly attributed to premating sexual selection (Meagher *et al.*, 2000; Joron & Brakefield, 2003), reflecting inbreeding depression in the expression of secondary sexual traits and consequent mating success (Van Oosterhout *et al.*, 2003; Reid *et al.*, 2005; Mariette *et al.*, 2006; Ala-Honkola *et al.*, 2009; Pölkki *et al.*, 2012). However, inbreeding depression in male reproductive success could also reflect inbreeding depression in traits affecting post-copulatory or post-pollination sexual selection. Foremost among these may be gametic traits. In plants and animals, pollen and sperm performance can, respectively, predict reproductive fitness (Snow & Spira, 1991, 1996; Birkhead & Møller, 1998; Pizzari & Parker, 2009). However, there has been no synthesis of the degree to which inbreeding, referring to the coefficient of inbreeding (f) of a focal male, might be expected to or does affect male's gametic performance and hence create inbreeding depression in male reproductive success and fitness through post-copulatory or post-pollination sexual selection.

Inbreeding depression is widely attributed to the increased genome-wide homozygosity that results from inbreeding (Lynch & Walsh, 1998). Such increased homozygosity can decrease fitness by increasing the expression of deleterious recessive alleles and hence exposing genetic load (partial dominance hypothesis) and/or by reducing expression of heterozygote advantage (overdominance hypothesis, Charlesworth & Charlesworth, 1987; Lynch & Walsh, 1998; Roff, 2002; Charlesworth & Willis, 2009). Inbreeding depression is therefore understood to be a property of diploid entities where phenotypes depend on genetic dominance effects. More explicitly, inbreeding depression requires directional dominance such that the phenotype of heterozygotes differs from the mean of the two homozygotes (Charlesworth & Charlesworth, 1999). This situation is likely to apply to fitness-related traits that are under strong directional selection, and hence where deleterious dominant alleles will be rapidly eliminated but deleterious recessive alleles will be maintained at low frequencies (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Traits that are closely related to fitness might consequently show higher inbreeding depression than traits that are less closely related to fitness (which might be under stabilizing rather than directional selection, Lynch & Walsh, 1998; DeRose & Roff, 1999; Cotton *et al.*, 2004). Because sperm- and pollen-performance-related traits can influence fitness (Snow & Spira, 1991, 1996; Birkhead & Møller, 1998; Pizzari & Parker, 2009) and are at least partly under diploid genetic control through gene expression in the focal male, inbreeding depression might be expected.

However, individual sperm and pollen cells carry haploid genomes that result from chromosomal segregation (and crossing-over) during meiosis (Stephenson *et al.*, 2001; Higginson & Pitnick, 2011). Because sperm and pollen cells are haploid, genetic dominance effects cannot exist and inbreeding depression in gametic traits that result from haploid gene expression cannot arise through currently understood mechanisms. The degree to which male gametic traits could show inbreeding depression will therefore depend on the degree to which they are under haploid vs. diploid genetic control, as well as on segregating genetic loads. In plants, the haploid pollen genome is expressed from microsporogenesis (meiosis giving rise to pollen grains) until the pollen tube enters the ovule (Mascarenhas, 1993; Stephenson *et al.*, 2001). Newly formed pollen grains (microgametophytes) require expression of their own haploid genome to develop, germinate and grow, and aspects of their performance (speed of germination and pollen growth rate) depend on their haploid genotype (Sari-Gorla & Frova, 1997). In animals, expression of haploid genes in sperm is generally thought to be rather limited (Pizzari & Parker, 2009), but recent studies suggest that part of the haploid genome might be expressed and influence sperm function (Joseph & Kirkpatrick, 2004; Immler, 2008; Pizzari & Foster, 2008). The expression of haploid sperm and pollen genes allows selection at the individual gamete level, creating competition between individual sperm or pollen genes within a single male as well as among males (Parker & Begon, 1993; Stephenson *et al.*, 2001; Immler, 2008). Because the relative roles of diploid vs. haploid genetic control in male gametic performance are poorly known, it is not immediately clear to what degree inbreeding depression in male gametic performance should be expected.

If inbreeding depression in male gametic performance, defined as a decrease in the performance of a male's sperm or pollen with increasing f of the focal male, did arise, it could have profound implications for mating system evolution and conservation biology. First, inbreeding depression in male gametic performance could create sex-specific inbreeding depression in fitness. Although the overall evidence is still equivocal, recent studies on animal species suggest that inbreeding depression in fitness can be greater in males than in females (Enders & Nunney, 2010; Janicke *et al.*, 2013). Sex-specific inbreeding depression implies that selection against deleterious recessive alleles can differ between the sexes (assuming equal directional

dominance; Agrawal, 2011; Mallet & Chippindale, 2011), which could cause sex-specific purging (Whitlock & Agrawal, 2009) and potentially create sex-specific evolutionary trajectories. Sex-specific inbreeding depression could also influence mating system evolution. For example in plants, differential inbreeding depression in male and female fitness components could facilitate the evolution of stable mixed mating systems where both selfing and outcrossing are maintained (Rausher & Chang, 1999).

Second, inbreeding depression in male gametic performance could influence the coevolutionary dynamics of polyandry (female mating with multiple males during a single reproductive episode) and inbreeding. Polyandry is widely hypothesized to have evolved as a means of avoiding inbreeding and consequent inbreeding depression (Jennions & Petrie, 2000; Tregenza & Wedell, 2000; Zeh & Zeh, 2001; Barrett, 2002; Simmons, 2005). However, regardless of its ultimate cause, polyandry causes coexistence of sperm or pollen of more than one male at or near the site of fertilization, creating competition for the fertilization of the eggs or ovules (Parker, 1970; Mulcahy & Mulcahy, 1987). By creating gamete competition, polyandry might increase the magnitude of inbreeding depression in male fitness in situations where inbreeding reduces a male's gametic performance in competition for fertilization. This could in turn increase the evolutionary benefit of inbreeding avoidance, potentially altering the magnitude of selection on polyandry.

Third, inbreeding depression in pollen performance could have synergistic interactions with other factors that shape plant mating systems. For example, several studies have shown that inbreeding, and specifically selfing, could reduce the quantity of pollen available for outcrossing (known as 'pollen discounting', Wells, 1979; Goodwillie *et al.*, 2005; Charlesworth, 2006). Inbreeding depression in pollen performance could additionally reduce the quality of pollen available for outcrossing, potentially leading to a further reduction in fertilization success than typically considered under pollen discounting. This compounding cost of inbreeding could make partial selfing (i.e. mixed mating systems) harder to maintain in nature.

Fourth, inbreeding depression in male gametic performance could affect the efficacy of artificial breeding programmes. Artificial insemination is widely used as a conservation tool to allow reproduction between individuals that are unable to achieve natural reproduction, to exchange genetic material between captive and wild populations and to manage reproduction to minimize inbreeding and inbreeding depression (Wildt *et al.*, 1995). In animals, sperm characteristics can substantially influence the success of artificial inseminations (Blanco *et al.*, 2009). Inbreeding depression in sperm performance could therefore reduce the efficacy of planned artificial inseminations. In plants, reduced pollination ability following inbreeding or selfing can substantially reduce crop yield, compromise plant breeding programmes or pose greater requirements for efficacious pollination (Allard, 1999). Inbreeding depression in pollen performance could underlie such negative effects, potentially leading to species extinction (Brown & Caligari, 2013).

Here, we first consider the possible mechanisms through which inbreeding could impact male gametic performance. We then assess the evidence that inbreeding depression in male gametic performance is a general phenomenon by conducting a literature review and prospective meta-analysis of studies that estimate the magnitude of inbreeding load in sperm- and pollen-performance-related traits. We discuss the implications of our results and requirements for future studies.

Potential mechanisms of inbreeding depression in male gametic performance

Sperm performance

Mature haploid spermatozoa (i.e. sperm cells) develop from diploid germ cells in a multistep, organized and synchronized process of meiosis, named spermatogenesis (Roosen-Runge, 1962; de Franca *et al.*, 2008). During spermatogenesis, germ cells interact and keep constant contact with somatic cells (e.g. cyst cells in *Drosophila*, Sertoli cells in mammals; White-Cooper *et al.*, 2009), which produce essential androgens and gonadotrophins (e.g. testosterone produced by Leydig cells in mammals) and shape spermatogenesis (Knobil & Neil, 1994; Walker, 2011). Homozygosity in inbred males could potentially affect all stages of this continuous process through multiple nonexclusive mechanisms. For example, before the onset of meiosis, deleterious mutations could be expressed in the relatively homozygous diploid germ cells of inbred males, potentially impacting the quantity, phenotype and performance of subsequently formed sperm cells. During spermatogenesis, diploid somatic cells play a major role as shown in knockout mice where spermatogenesis is impaired in the enforced absence of testosterone or androgen receptors (De Gendt *et al.*, 2004), following Leydig hyperplasia (Robertson *et al.*, 1999), or reduced androgen receptors in Sertoli cells (Chang *et al.*, 2004). Consequently, increased homozygosity at loci regulating the synthesis and functioning of androgens, androgen receptors and testis somatic support cells could cause inbreeding depression in the quantity (e.g. sperm number) or quality (e.g. sperm swimming velocity) of an inbred male's mature haploid sperm cells (see Literature Review section for description and definition of sperm traits).

During and following spermatogenesis, male homozygosity could also indirectly reduce haploid sperm performance via effects on the sperm environment. Sperm

are released in seminal fluid, whose properties strongly influence sperm characteristics such as velocity and motility (Chapman, 2001; Froman, 2003) and capacitation (a maturation process where sperm acquire the ability to fertilize an oocyte in mammals; Sanz *et al.*, 1993). Seminal fluid also influences sperm storage and nourishment in the female tract (Gillott, 1996; Wolfner, 2002) and sperm defence against chemical attacks (Alexander & Anderson, 1987; Chapman, 2001). The composition and allocation of seminal fluid is consequently under strong selection (Cornwallis & O'Connor, 2009; Wigby *et al.*, 2009). As seminal fluid is created by diploid gene expression in males, inbreeding depression in sperm performance could arise as a consequence of impeded functioning of sexual organs of inbred males.

Finally, there is now evidence that some haploid gene expression can occur in sperm (e.g. in house mice *Mus musculus*, reviewed in Joseph & Kirkpatrick, 2004). Because inbreeding depression stems from genetic dominance effects, inbreeding depression in resulting haploid phenotypes is not expected (see Introduction). However, fascinatingly, haploid gene products have been shown to be shared among developing sperm in mice, making sperm phenotypes effectively diploid due to interactions between multiple sperm cells (Braun *et al.*, 1989; Joseph & Kirkpatrick, 2004). Selection might then act on diploid phenotypes that are jointly expressed by haploid sperm cells, potentially resulting in inbreeding depression.

Pollen performance

Flowering plants do not have specified germ lines during initial vegetative growth. Instead, at the onset of reproduction, a group of cells inside the meristem differentiate into anthers. Sporogenesis occurs inside the anthers, creating microspore mother cells that are surrounded by several layers of sterile cells including nutritive cells that constitute the tapetum layer (Shivanna & Johri, 1985; Furness *et al.*, 2002). Each microspore mother cell, which contains a diploid genome, undergoes meiosis to produce four haploid daughter cells that are initially held together as a tetrad. Post-meiotic processes allow the individual microspores to separate, undergo a mitotic division and form two daughter cells of uneven sizes, with the smaller generative cell enclosed inside the larger vegetative cell (Shivanna & Johri, 1985; Twell, 2011). The generative cell eventually divides through mitosis into two cells (referred to as 'sperm' cells), and following successful pollination, the vegetative cell germinates and grows a pollen tube that transports the sperm to the egg inside the ovule.

Multiple stages in pollen development are influenced by the diploid genome in the sporophytes. For example, tapetal tissues surrounding the pollen mother cells and developing pollen grains provide the grains with metabolites, enzymes, nutrients, pollen wall synthesis and pollen coat deposition (Shivanna & Johri, 1985; Willing *et al.*, 1988; Piffanelli *et al.*, 1998; Parish & Li, 2010). Mutations that disrupt the tapetum development or its functions can lead to abnormally small pollen grains with a slower tube growth rate than normal pollen and, in some cases, even male sterility (Li *et al.*, 2006; Guo & Liu, 2012; Liu & Fan, 2013). In addition, tapetal cells are also involved in synthesizing sporopollenin, a major ingredient for the pollen grain outer wall (Ariizumi & Toriyama, 2011) that is important for structural support as well as for grain viability. As pollen grains mature, the tapetum layer eventually undergoes programmed cell death (Scott *et al.*, 2004) and releases organelles, some of which are incorporated into the pollen coat (Li *et al.*, 2006; Liu & Fan, 2013). Abnormal tapetum development or persistence can therefore cause abnormal pollen walls (Dou *et al.*, 2011; Li *et al.*, 2011), potentially impeding the initial hydration and tube germination of the pollen grains on the stigmatic surface (Dickinson & Elleman, 1985; Blackmore *et al.*, 2007; Ariizumi & Toriyama, 2011; Liu & Fan, 2013). Pollen grains are therefore influenced by the diploid sporophytic genome in both the content inside the vegetative cell and the materials on the pollen wall surface. Hence, there are ample opportunities for inbreeding depression in diploid cells to affect the performance of the haploid pollen grains beyond solely any inbreeding depression in pollen quantity that might arise.

Inbreeding depression due to reduced genetic diversity

A different mechanism through which the coefficient of inbreeding (f) of a focal male could potentially affect sperm or pollen performance involves reduced genetic diversity among individual sperm cells or pollen grains within a male. During gametogenesis, both meiotic recombination and *de novo* recombination produce genetic diversity among gametes (Till-Bottraud *et al.*, 2005; Wang *et al.*, 2012). Genetic diversity within germ and mother cells is expected to decrease with increasing f, meaning that more inbred males might produce less genetically diverse gametes. Because genetic diversity is a fundamental source of phenotypic diversity and can influence fitness (Reed & Frankham, 2003; Hughes *et al.*, 2008), gamete pools that contain reduced genetic diversity might impede a male's gametic performance and ultimate fertilization success. For example in animals, because the optima of different sperm traits (e.g. size and longevity) cannot always be achieved simultaneously within single sperm, males producing phenotypically diverse sperm can have a fertilization advantage (Immler *et al.*, 2007; Pizzari & Parker, 2009). To the degree that genetic diversity among individual sperm (i.e. intra-male variance) could create phenotypic diversity, inbred males could show reduced performance in sperm competition. This potential mechanism under-

lying inbreeding depression in male gametic performance, and hence fitness, differs from those described previously or considered more widely because it does not require genetic dominance effects on phenotype.

In summary, given the multiple potential mechanisms through which inbreeding could affect male gametic quantity and quality, there would seem to be considerable scope for inbreeding depression in male gametic performance and hence fertilization success.

Literature review

To assess the evidence that inbreeding depression can affect male gametic performance, we undertook a literature review of studies that estimated inbreeding loads in relevant traits. We first explain and justify the metrics of sperm and pollen performance considered (summarized in Table 1).

Sperm performance

It is widely accepted that sperm performance depends on sperm quantity (e.g. ejaculate size and percentage of motile sperm; Table 1) and quality (e.g. sperm swimming velocity and longevity; Parker, 1998; Pizzari & Birkhead, 2000; Snook, 2005; Pizzari *et al.*, 2008; Pizzari & Parker, 2009; Table 1). Sperm swimming velocity predicts fertilization success in domestic (Birkhead *et al.*, 1999; Donoghue *et al.*, 1999; Burness *et al.*, 2004), captive (Levitan, 2000; Burness *et al.*, 2004) and wild populations (Gage *et al.*, 2004; Malo *et al.*, 2005; Casselman *et al.*, 2006; Gasparini *et al.*, 2010), as do motility (Hunter & Birkhead, 2002; Denk *et al.*, 2005; Malo *et al.*, 2005) and longevity (Levitan, 2000), at least in some species. The outcome of sperm competition might also be influenced by sperm number (Martin *et al.*, 1974; Parker, 1982; Gage & Morrow, 2003; Table 1) and by morphological traits including total sperm length, head length and flagellum length (Gomendio & Roldan, 2008; Lüpold *et al.*, 2009) and their ratios (Humphries *et al.*, 2008) although sperm number and morphology can trade off in complex ways (Snook, 2005; Pizzari & Parker, 2009). Additionally, sperm oxidative damage (i.e. damage due to oxidative stress, the imbalance between reactive oxygen species and the antioxidant response, Sies, 1991) has recently been identified as a major impediment to sperm performance in several species (Aitken, 1999; Tremellen, 2008; Pike *et al.*, 2010; Losdat *et al.*, 2011).

Further metrics of a male's sperm performance involve the number or proportion of eggs fertilized (Table 1), measuring male fertilizing efficiency in a noncompetitive context (e.g. strictly monogamous species) or sperm competitive ability in a competition situation (Birkhead, 1998). Given competition, both the proportion of offspring fathered by the first male to mate with a female (P1, 'sperm defence') and the proportion of offspring fathered by the second male to mate with a female (P2, 'sperm offence') can be measured (Boorman & Parker, 1976; Table 1).

Pollen performance

Similar to sperm, the quantity and quality of pollen can both be important for male reproductive success (Aizen & Harder, 2007). The sessile nature of plants makes the delivery of pollen grains from the source anthers to conspecific target stigmas an indirect process that relies on external pollination agents (e.g. insects, wind). The efficacy of these agents partially depends on the quantity of pollen they collect and deliver to the intended targets (Ashman, 1998). As a large proportion of exported pollen grains never reach conspecific stigmas (Holsinger & Thomson, 1994), the quantity of pollen produced, which is influenced by both the number of staminate flowers and the number of pollen grains produced per flower, is an important predictor of individual fertilization success (e.g. Muchhala & Thomson, 2010; Perry & Dorken, 2011; De Cauwer *et al.*, 2012).

Once pollen grains are successfully delivered to the stigmas, pollen quality becomes important. Individual pollen grains often encounter conspecific grains on the same stigma, and competitive ability becomes the key factor in determining relative siring success. As faster-growing pollen tubes can reach the eggs earlier, relative pollen tube growth rate is key (Pasonen *et al.*, 2001; Lankinen & Armbruster, 2007). Appropriate metrics of male gametic performance in plants therefore include total pollen production, pollen production per flower, size of pollen grains, speed of pollen germination and pollen tube growth rate (Table 1).

Results

We searched for studies that estimated inbreeding depression in sperm and pollen performance traits through Google Scholar and Web of Science using different combinations of the keywords: 'relatedness', 'inbreeding', 'inbreeding depression', 'sperm', 'sperm quality', 'sperm performance', 'paternity', 'male', 'fertility', 'pollen', 'pollen tube growth', 'pollen size', 'pollen volume' and 'pollen production'. We then checked the reference lists of every article found through the initial searches for additional relevant articles. Through this process, we identified 51 studies (30 animal and 21 plant studies) that considered 35 gametic traits, totalling 183 pollen or sperm study traits in 37 species (24 animals and 13 plants, Table 2). The animal species comprised 16 vertebrates and eight invertebrates. Of the 51 studies, 46 used captive populations (28 animals and 18 plants) whereas only five used wild populations (two animals and three plants). Furthermore, both these animal studies (Wildt *et al.*, 1987; Gage *et al.*, 2006) used microsatellite heterozygosity rather than pedigree-based estimates of inbreeding, and the three plant

Table 1 List of sperm and pollen performance traits considered (in alphabetical order). Definitions follow the studies included in the literature review and therefore pertain to those specific studies.

Male gametic trait	Definition	References
Sperm traits		
Fertility	Total number of offspring sired by a male	Malo *et al.* (2006); Michalczyk *et al.* (2010)
Percentage of morphologically normal spermatozoa	Percentage of sperm that do not show physical abnormalities	Gomendio *et al.* (2000); van Eldik *et al.* (2006)
Percentage of motile sperm	Percentage of sperm that move/are alive	Birkhead & Møller (1998); Pizzari & Parker (2009)
Percentage of normal sperm	Percentage of sperm that show no abnormalities in any measured trait	Gomendio *et al.* (2000)
Percentage of oxidative DNA damage	Percentage of sperm with a high level of DNA fragmentation	Ruiz-Lòpez *et al.* (2010)
Percentage of spermatozoa with progressive motility	Percentage of sperm that move forward normally, also known as sperm viability	Gomendio *et al.* (2000); van Eldik *et al.* (2006)
Proportion of offspring sired (P1 sperm defence)	Percentage of offspring sired by the first (of two) males to mate with a given female	Hughes (1997); Simmons (2011)
Proportion of offspring sired (P2 sperm offence)	Percentage of offspring sired by the second (of two) males to mate with a given female	Hughes (1997); Simmons (2011)
Proportion of offspring sired (P1 and P2 combined)	Percentage of offspring sired by a male in a sperm competition situation	Hughes (1997); Simmons (2011)
Sperm concentration	Total number of sperm cells for a given volume of ejaculate	Wildt *et al.* (1982); van Eldik *et al.* (2006)
Sperm morphological measures (total length, head length, midpiece length, flagellum length, head volume)	Within-male mean length (μm) of a given part of sperm. Head volume = π/6*(head length)^3	Mehlis *et al.* (2012)
Sperm morphological ratios (head/flagellum and midpiece/flagellum)	Within-male mean ratio value of sperm parts	Mehlis *et al.* (2012)
Sperm morphometry: variability in sperm length	Intra-male coefficient of variation in total sperm length	Michalczyk *et al.* (2010)
Sperm volume	Ejaculate volume	Maximini *et al.*, 2011;
Total sperm counts	Total number of sperm in an ejaculate	Wildt *et al.* (1982); Zajitschek & Brooks (2010)
Pollen traits		
Days with staminate flowers	Total number of days on which staminate (male) flowers were produced	Hayes *et al.* (2005b)
Multiplicative male fitness	Product of staminate flowers per plant, pollen number per flower and *in vitro* pollen tube growth rate	Stephenson *et al.* (2004); Hayes *et al.* (2005a)
Number of pollen grains	Number of pollen grains per stigma or flower	Aizen *et al.* (1990); Melser *et al.* (1999)
Number of pollen tubes per flower	Number of growing pollen tubes per flower	Montalvo (1992)
Number of staminate flowers per plant	Total number of staminate (male) flowers produced	Jóhannsson *et al.* (1998); Stephenson *et al.* (2001)
Percentage of pollen germinating	Percentage of pollen that germinate in controlled conditions	Austerlitz *et al.* (2012)
Pollen diameter	Mean pollen diameter (μm) per individual	Hayes *et al.* (2005b)
Pollen tube length	Mean pollen tube length (μm) measured *in vitro*	Aizen *et al.* (1990); Hayes *et al.* (2005b)
Pollen viability	Proportion of alive pollen (vs. dead pollen) measured through staining method	Hayes *et al.* (2005b)
Siring success	Proportion of seeds sired following pollination by a mixture of inbred/outbred pollen	Jóhannsson *et al.* (1998); Austerlitz *et al.* (2012)

studies used wild-collected seeds that were then inbred, grown and pollen traits studied in constrained conditions. There is therefore no study that estimated inbreeding load in sperm or pollen performance based on explicit estimates of f where focal males lived under natural conditions. The large diversity of gametic traits measured across studies and the limited and unbalanced sample size per trait (Table 2) precluded a formal quantitative meta-analysis that corrected for phylogeny and publication bias. We therefore conducted a 'prospective' meta-analysis that simply compiles estimated effects (Simmons & Moore, 2009).

Table 2 Summary of studies estimating inbreeding depression in sperm and pollen traits.

Gametic trait/species	Conditions	Design	Inbreeding estimation	Sample size	Range of *f*	Reported effect	−*B*	References
Sperm traits								
Fertility (number of offspring)†								
Physa acuta	Lab/captive	Comparison	Breeding design	194	0–0.5	–	0.53†	Janicke *et al.* (2013)
Drosophila melanogaster	Lab/captive	Comparison	Breeding design	94	0–0.25	0	−0.09	Ala-Honkola *et al.* (2013)
D. melanogaster	Lab/captive	Comparison	Breeding design	96	0–0.5	0	0.45	Ala-Honkola *et al.* (2013)
Tribolium castaneum	Lab/captive	Comparison	Breeding design	29	0–0.83	0	−0.06	Michalczyk *et al.* (2010)
Drosophila simulans	Lab/captive	Comparison	Breeding design	200	0–0.25	–	−0.83	Okada *et al.* (2011)
D. melanogaster	Lab/captive	Comparison	Breeding design	1050	0–0.79	–	−0.09	Pedersen *et al.* (2011)
D. melanogaster	Lab/captive	Regression	Breeding design	X	0.02–0.25	0	1.22	Robinson *et al.* (2009)
Peromyscus leucopus	Lab/captive	Regression	Breeding design	59	0.09–0.17	0	−1.88	Malo *et al.* (2010)
P. leucopus	Lab/captive	Regression	Breeding design	59	0.1–0.17	–	−0.38	Malo *et al.* (2010)
Percentage of morphologically normal spermatozoa*								
Canis lupus baileyi	Lab/captive	Regression	Breeding design	55	0.11–0.61	–	X	Asa *et al.* (2007)
Equus ferus	Lab/captive	Regression	Microsatellites	104	NA	0	NA	Aurich *et al.* (2003)
E. ferus	Lab/captive	Regression	Pedigree	285	0–0.26	–	−1.9	van Eldik *et al.* (2006)
Gazella cuvieri	Lab/captive	Regression	Pedigree	14	0.06–0.23	–	X	Gomendio *et al.* (2000)
Gazella dama	Lab/captive	Regression	Pedigree	17	0–0.31	0	X	Gomendio *et al.* (2000)
Gazella dorcas	Lab/captive	Regression	Pedigree	19	0–0.08	0	X	Gomendio *et al.* (2000)
Percentage of motile sperm*								
C. lupus baileyi	Lab/captive	Regression	Breeding design	55	0.11–0.61	–	X	Asa *et al.* (2007)
P. leucopus	Lab/captive	Regression	Breeding design	59	0.09–0.17	–	−1.66	Malo *et al.* (2010)
E. ferus	Lab/captive	Regression	Microsatellites	104	NA	0	NA	Aurich *et al.* (2003)
Mesocricetus auratus	Lab/captive	Comparison	Microsatellites	20	NA	0	NA	Fritzsche *et al.* (2006)
E. ferus	Lab/captive	Regression	Pedigree	285	0–0.26	–	−0.44	van Eldik *et al.* (2006)
G. cuvieri	Lab/captive	Regression	Pedigree	14	0.06–0.23	–	X	Gomendio *et al.* (2000)
G. dama	Lab/captive	Regression	Pedigree	17	0–0.31	0	X	Gomendio *et al.* (2000)
G. dorcas	Lab/captive	Regression	Pedigree	19	0–0.08	0	X	Gomendio *et al.* (2000)
Myliobatis australis	Lab/captive	Regression	Pedigree	317	0.01–0.11	X	X	Maximini *et al.* (2011)
M. australis	Lab/captive	Regression	Pedigree	398	0.01–0.13	–	X	Maximini *et al.* (2011)
G. cuvieri	Lab/captive	Regression	Pedigree	11	0.06–0.23	–	X	Roldan *et al.* (1998)
Canis lupus	Lab/captive	Comparison	Pedigree	18	0–0.26	0	−4.01	Wildt *et al.* (1982)
Gazella dama mhorr	Lab/captive	Regression	Pedigree	22	0.27–0.40	0	X	Ruiz-Lòpez *et al.* (2012)
Lynx pardinus	Lab/captive	Regression	Heterozygosity	20	NA	0	NA	Ruiz-Lòpez *et al.* (2012)
D. simulans	Lab/captive	Comparison	Breeding design	55	0–0.25	0	0.63	Okada *et al.* (2011)
Percentage of normal sperm*								
Panthera leo	Wild	Comparison	Allozymes	25	NA	–	NA	Wildt *et al.* (1987)
Oryctolagus cuniculus	Wild	Regression	Microsatellites	91	NA	–	NA	Gage *et al.* (2006)
E. ferus	Lab/captive	Regression	Pedigree	285	0–0.26	–	−2.88	van Eldik *et al.* (2006)
G. cuvieri	Lab/captive	Regression	Pedigree	14	0.06–0.23	–	X	Gomendio *et al.* (2000)
G. dama	Lab/captive	Regression	Pedigree	17	0–0.31	0	X	Gomendio *et al.* (2000)
G. dorcas	Lab/captive	Regression	Pedigree	19	0–0.08	0	X	Gomendio *et al.* (2000)
Peromyscus polionotus	Lab/captive	Regression	Pedigree	93	0.1–0.5	–	X	Margulis & Walsh (2002)
G. cuvieri	Lab/captive	Regression	Pedigree	11	0.06–0.23	–	X	Roldan *et al.* (1998)
Percentage of oxidative DNA damage*								
D. simulans	Lab/captive	Comparison	Breeding design	200	0–0.25	–	−0.37	Okada *et al.* (2011)
Ovies aries	Lab/captive	Comparison	Pedigree	20	0–0.25	–	−4.96	Petrovic *et al.* (2013)
Gazella spp.	Lab/captive	Regression	Pedigree	49	0–0.31	–	X	Ruiz-Lòpez *et al.* (2010)

Table 2 (*Continued*)

Gametic trait/species	Conditions	Design	Inbreeding estimation	Sample size	Range of *f*	Reported effect	−*B*	References
Percentage of spermatozoa with progressive motility*								
E. ferus	Lab/captive	Regression	Pedigree	285	0–0.26	0	−3.46	van Eldik *et al.* (2006)
G. cuvieri	Lab/captive	Regression	Pedigree	14	0.06–0.23	–	X	Gomendio *et al.* (2000)
G. dama	Lab/captive	Regression	Pedigree	17	0–0.31	0	X	Gomendio *et al.* (2000)
G. dorcas	Lab/captive	Regression	Pedigree	19	0–0.08	0	X	Gomendio *et al.* (2000)
Proportion of offspring sired (P1 sperm defence)†								
Gryllodes supplicans	Lab/captive	Comparison	Breeding design	14	0–0.25	–	X	Stockley (1999)
D. melanogaster	Lab/captive	Comparison	Breeding design	X	X	–	−0.29	Hughes (1997)
T. castaneum	Lab/captive	Comparison	Breeding design	164	0–0.83	–	−0.32	Michalczyk *et al.* (2010)
D. melanogaster	Lab/captive	Regression	Breeding design	X	0.02–0.25	0	−0.67	Robinson *et al.* (2009)
Proportion of offspring sired (P2 sperm offence)†								
D. melanogaster	Lab/captive	Comparison	Breeding design	94	0–0.25	0	0	Ala-Honkola *et al.* (2013)
D. melanogaster	Lab/captive	Comparison	Breeding design	96	0–0.5	–	−0.075	Ala-Honkola *et al.* (2013)
Rhizoglyphus robini	Lab/captive	Comparison	Breeding design	100	0–0.25	–	−3.09	Konior *et al.* (2005)
T. castaneum	Lab/captive	Comparison	Breeding design	164	0–0.83	–	−0.52	Michalczyk *et al.* (2010)
D. melanogaster	Lab/captive	Comparison	Breeding design	X	X	–	−0.40	Hughes (1997)
D. melanogaster	Lab/captive	Regression	Breeding design	X	0.02–0.25	0	−0.22	Robinson *et al.* (2009)
Proportion of offspring sired (P1 and P2 merged)†								
P. acuta	Lab/captive	Comparison	Breeding design	194	0–0.5	–	−1.81	Janicke *et al.* (2013)
D. melanogaster	Lab/captive	Comparison	Breeding design	94	0–0.25	0	0	Ala-Honkola *et al.* (2013)
D. melanogaster	Lab/captive	Comparison	Breeding design	96	0–0.5	–	−0.075	Ala-Honkola *et al.* (2013)
Teleogryllus oceanicus	Lab/captive	Comparison	Breeding design	77	0–0.25	–	−1.28	Simmons (2011)
Poecilia reticulata	Lab/captive	Comparison	Breeding design	32	0–0.59	–	−2.36	Zajitschek *et al.* (2009)
P. reticulata	Lab/captive	Comparison	Breeding design	9	0–0.25	0	0.37	Zajitschek *et al.* (2009)
M. auratus	Lab/captive	Comparison	Microsatellites	20	NA	–	NA	Fritzsche *et al.* (2006)
Sperm concentration*								
E. ferus	Lab/captive	Regression	Microsatellites	104		0	NA	Aurich *et al.* (2003)
E. ferus	Lab/captive	Regression	Pedigree	285	0–0.26	0	−0.8	van Eldik *et al.* (2006)
M. australis	Lab/captive	Regression	Pedigree	317	0.01–0.11	0	X	Maximini *et al.* (2011)
C. lupus	Lab/captive	Comparison	Pedigree	18	0–0.26	–	−7.45	Wildt *et al.* (1982)
Sperm morphometry: total sperm length*								
D. melanogaster	Lab/captive	Comparison	Breeding design	44	0–0.25	0	−0.05	Ala-Honkola *et al.* (2013)
D. melanogaster	Lab/captive	Comparison	Breeding design	43	0–0.5	0	−0.02	Ala-Honkola *et al.* (2013)
T. castaneum	Lab/captive	Comparison	Breeding design	30	0–0.83	0	−0.01	Michalczyk *et al.* (2010)
Sperm morphometry: flagellum length*								
Gasterosteus aculeatus R.	Lab/captive	Comparison	Breeding design	23	0–0.5	0	−0.15	Mehlis *et al.* (2012)
G. cuvieri	Lab/captive	Regression	Pedigree	11	0.06–0.23	–	X	Roldan *et al.* (1998)
Sperm morphometry: head length*								
G. aculeatus R.	Lab/captive	Comparison	Breeding design	23	0–0.5	0	−0.09	Mehlis *et al.* (2012)
G. cuvieri	Lab/captive	Regression	Pedigree	11	0.06–0.23	0	X	Roldan *et al.* (1998)
Sperm morphometry: head volume*								
G. aculeatus R.	Lab/captive	Comparison	Breeding design	23	0–0.5	0	−0.19	Mehlis *et al.* (2012)
Sperm morphometry: head- or midpiece-to-flagellum ratio*								
G. aculeatus R.	Lab/captive	Comparison	Breeding design	23	0–0.5	0	−0.15	Mehlis *et al.* (2012)
G. cuvieri	Lab/captive	Regression	Pedigree	11	0.06–0.23	0	X	Roldan *et al.* (1998)
Sperm morphometry: midpiece length*								
G. cuvieri	Lab/captive	Regression	Pedigree	11	0.06–0.23	0	X	Roldan *et al.* (1998)
Sperm morphometry: total sperm length*								
D. melanogaster	Lab/captive	Comparison	Breeding design	44	0–0.25	0	−0.05	Ala-Honkola *et al.* (2013)
D. melanogaster	Lab/captive	Comparison	Breeding design	43	0–0.5	0	−0.02	Ala-Honkola *et al.* (2013)
T. castaneum	Lab/captive	Comparison	Breeding design	30	0–0.83	0	−0.01	Michalczyk *et al.* (2010)
G. cuvieri	Lab/captive	Regression	Pedigree	11	0.06–0.23	0	X	Roldan *et al.* (1998)

Table 2 (*Continued*)

Gametic trait/species	Conditions	Design	Inbreeding estimation	Sample size	Range of *f*	Reported effect	−*B*	References
Sperm morphometry: variability in sperm length*								
T. castaneum	Lab/captive	Comparison	Breeding design	30	0–0.83	–	−0.40	Michalczyk *et al.* (2010)
Sperm volume*								
E. ferus	Lab/captive	Regression	Microsatellites	104	NA	0	NA	Aurich *et al.* (2003)
E. ferus	Lab/captive	Regression	Pedigree	285	0–0.26	+	1.93	van Eldik *et al.* (2006)
M. australis	Lab/captive	Regression	Pedigree	317	0.01–0.11	0	X	Maximini *et al.* (2011)
M. australis	Lab/captive	Regression	Pedigree	398	0.13–0.14	–	X	Maximini *et al.* (2011)
G. cuvieri	Lab/captive	Regression	Pedigree	11	0.06–0.23	–	X	Roldan *et al.* (1998)
C. lupus	Lab/captive	Comparison	Pedigree	18	0–0.26	0	−2.0	Wildt *et al.* (1982)
Total sperm counts*								
Callosobruchus maculatus	Lab/captive	Comparison	Breeding design	165	0–0.25	–	−1.15	Fox *et al.* (2012)
G. aculeatus R.	Lab/captive	Comparison	Breeding design	46	0–0.5	0	−0.11	Mehlis *et al.* (2012)
P. reticulata	Lab/captive	Comparison	Breeding design	61	0–0.25	–	−1.4	Zajitschek & Brooks (2010)
P. reticulata	Lab/captive	Comparison	Breeding design	62	0–0.59	–	−1.34	Zajitschek & Brooks (2010)
P. reticulata	Lab/captive	Comparison	Breeding design	40	0–0.38	0	−0.69	Zajitschek & Brooks (2010)
P. reticulata	Lab/captive	Comparison	Breeding design	62	0–0.5	–	−1.7	Zajitschek & Brooks (2010)
E. ferus	Lab/captive	Regression	Microsatellites	104	NA	0	NA	Aurich *et al.* (2003)
M. auratus	E/lab-wild	Comparison	Microsatellites	20	NA	0	NA	Fritzsche *et al.* (2006)
P. polionotus	Lab/captive	Regression	Pedigree	93	0.1–0.5	–	X	Margulis & Walsh (2002)
M. australis	Lab/captive	Regression	Pedigree	317	0.01–0.11	–	X	Maximini *et al.* (2011)
M. australis	Lab/captive	Regression	Pedigree	398	0.01–0.13	–	X	Maximini *et al.* (2011)
C. lupus	Lab/captive	Comparison	Pedigree	18	0–0.26	–	−6.4	Wildt *et al.* (1982)
Pollen traits								
Days with staminate flowers‡								
Cucurbita pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.25	–	−0.21	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.5	–	−0.06	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.75	–	−0.38	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.875	–	−0.2	Hayes *et al.* (2005b)
Multiplicative male fitness§								
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	224	0–0.5	–	−0.4	Stephenson *et al.* (2004)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	250	0–0.75	–	−0.75	Hayes *et al.* (2005a)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.25	–	−0.65	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.5	–	−0.66	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.75	–	−0.87	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.875	–	−0.66	Hayes *et al.* (2005b)
Number of pollen grains‡								
Echium vulgare	Lab/captive	Comparison	Breeding design	10	0–0.5	0	X	Melser *et al.* (1999)
Campanula rapunculoides	Lab/captive	Comparison	Breeding design	186	0–0.25	–	−0.42	Good-Avila & Stephenson (2003)
C. rapunculoides	Lab/captive	Comparison	Breeding design	153	0–0.5	–	−0.95	Good-Avila & Stephenson (2003)
C. rapunculoides	Lab/captive	Comparison	Breeding design	129	0–0.75	–	−1.33	Good-Avila & Stephenson (2003)

Table 2 (*Continued*)

Gametic trait/species	Conditions	Design	Inbreeding estimation	Sample size	Range of *f*	Reported effect	$-B$	References
Number of pollen tubes per flower‡								
Eichhornia paniculata	Lab/captive	Comparison	Breeding design	X	0–0.5	0	X	Cruzan & Barrett (1993)
E. vulgare	Lab/captive	Comparison	Breeding design	10	0–0.5	0	X	Melser *et al.* (1999)
Aquilegia caerulea	Wild	Comparison	Breeding design	132	0–0.5	0	−0.04	Montalvo (1992)
Amsinckia grandiflora	Lab/captive	Comparison	Breeding design	58	0–0.5	–	−2.74	Weller & Ornduff (1991)
Quercus ilex	Wild	Comparison	Breeding design	14	0–0.5	–	−0.8	Yacine & Bouras (1997)
Dianthus chinensis L.	Lab/captive	Comparison	Breeding design	160	0–0.5	–	−0.26	Aizen *et al.* (1990)
Mimulus guttatus	Lab/captive	Comparison	Breeding design	161	0–0.5	–	−0.56	Carr & Dudash (1995)
C. pepo ssp. *texana*	Lab/captive	Regression	Breeding design	174	0–0.875	–	−0.14	Hayes *et al.* (2004)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	250	0–0.75	0	−0.14	Hayes *et al.* (2005a)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	250	0–0.75	–	−0.3	Nelson Hayes *et al.* (2005)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	225	0–0.5	0	−0.06	Stephenson *et al.* (2004)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.25	–	−0.21	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.5	–	−0.15	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.75	–	−0.22	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.875	–	−0.16	Hayes *et al.* (2005b)
Number of staminate flowers per plant‡								
Collinsia heterophylla	Lab/captive	Comparison	Breeding design	174	0–0.5	–	−0.37	Lankinen & Armbruster (2007)
Silene latifolia	Lab/captive	Comparison	Breeding design	20	0–0.25	–	−0.24	Teixeira *et al.* (2008)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	272	0–0.5	–	−0.8	Jóhannsson *et al.* (1998)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.25	–	−0.68	Stephenson *et al.* (2001)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.5	–	−0.55	Stephenson *et al.* (2001)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.75	–	−0.59	Stephenson *et al.* (2001)
C. pepo ssp. *texana*	Lab/captive	Regression	Breeding design	238	0–0.875	–	−0.33	Hayes *et al.* (2004)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	250	0–0.75	–	−0.44	Hayes *et al.* (2005a)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.25	–	−0.25	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.5	–	−0.15	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.75	–	−0.37	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.875	–	−0.24	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	250	0–0.75	0	−0.48	Nelson Hayes *et al.* (2005)
Percentage of pollen germinating‡								
S. latifolia	Wild	Comparison	Breeding design	342	0–0.5	0	−0.19	Austerlitz *et al.* (2012)

Table 2 (*Continued*)

Gametic trait/species	Conditions	Design	Inbreeding estimation	Sample size	Range of *f*	Reported effect	−*B*	References
Pollen diameter‡								
C. pepo ssp. *texana*	Lab/captive	Regression	Breeding design	174	0–0.875	0	−0.02	Hayes *et al.* (2004)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.25	0	0	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.5	0	−0.02	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.75	0	−0.01	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.875	0	−0.01	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	250	0–0.75	0	0	Nelson Hayes *et al.* (2005)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	225	0–0.5	0	−0.06	Stephenson *et al.* (2004)
C. rapunculoides	Lab/captive	Comparison	Breeding design	186	0–0.25	–	−0.29	Good-Avila & Stephenson (2003)
C. rapunculoides	Lab/captive	Comparison	Breeding design	153	0–0.5	–	−0.02	Good-Avila & Stephenson (2003)
C. rapunculoides	Lab/captive	Comparison	Breeding design	129	0–0.75	–	−0.23	Good-Avila & Stephenson (2003)
Pollen tube length‡								
E. vulgare	Lab/captive	Comparison	Breeding design	10	0–0.5	–	−1.21	Melser *et al.* (1999)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.25	–	−0.27	Stephenson *et al.* (2001)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.5	–	−0.09	Stephenson *et al.* (2001)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.75	–	−0.17	Stephenson *et al.* (2001)
C. heterophylla	Lab/captive	Comparison	Breeding design	174	0–0.5	–	−0.33	Lankinen & Armbruster (2007)
Calluna vulgaris	Lab/captive	Comparison	Breeding design	41	0–0.5	0	0	Mahy & Jacquemart (1999)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	100	0–0.5	–	−0.8	Stephenson *et al.* (2004)
D. chinensis L.	Lab/captive	Comparison	Breeding design	160	0–0.5	–	−0.48	Aizen *et al.* (1990)
C. rapunculoides	Lab/captive	Comparison	Breeding design	134	0–0.25	–	−0.56	Good-Avila & Stephenson (2003)
C. rapunculoides	Lab/captive	Comparison	Breeding design	139	0–0.5	–	−0.26	Good-Avila & Stephenson (2003)
C. rapunculoides	Lab/captive	Comparison	Breeding design	121	0–0.75	–	−0.66	Good-Avila & Stephenson (2003)
E. paniculata	Lab/captive	Comparison	Breeding design	X	0–0.5	0	X	Cruzan & Barrett (1993)
C. pepo ssp. *texana*	Lab/captive	Regression	Breeding design	114	0–0.875	0	−0.10	Hayes *et al.* (2004)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	250	0–0.75	–	−0.15	Hayes *et al.* (2005a)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.25	–	−0.25	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.5	–	−0.37	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.75	–	−0.28	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	192	0–0.875	–	−0.27	Hayes *et al.* (2005b)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	250	0–0.75	–	−0.32	Nelson Hayes *et al.* (2005)

Table 2 (*Continued*)

Gametic trait/species	Conditions	Design	Inbreeding estimation	Sample size	Range of *f*	Reported effect	−*B*	References
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	272	0–0.5	–	−0.13	Jóhannsson *et al.* (1998)
Q. ilex	Wild	Comparison	Breeding design	14	0–0.5	–	X	Yacine & Bouras (1997)
E. vulgare	Lab/captive	Comparison	Breeding design	10	0–0.5	–	−1.21	Melser *et al.* (1999)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.25	–	−0.27	Stephenson *et al.* (2001)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.5	–	−0.09	Stephenson *et al.* (2001)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	X	0–0.75	–	−0.17	Stephenson *et al.* (2001)
Pollen viability‡								
E. vulgare	Lab/captive	Comparison	Breeding design	10	0–0.5	–	−0.07	Melser *et al.* (1999)
Siring success§								
S. latifolia	Lab/captive	Comparison	Breeding design	X	0–0.5	–	−0.79	Hauser & Siegismund (2000)
A. caerulea	Wild	Comparison	Breeding design	132	0–0.5	0	X	Montalvo (1992)
E. paniculata	Lab/captive	Comparison	Breeding design	X	0–0.5	–	X	Cruzan & Barrett (1993)
S. latifolia	Wild	Comparison	Breeding design	342	0–0.5	–	−0.94	Austerlitz *et al.* (2012)
C. pepo ssp. *texana*	Lab/captive	Comparison	Breeding design	272	0–0.5	–	−0.81	Jóhannsson *et al.* (1998)
C. vulgaris	Lab/captive	Comparison	Breeding design	49	0–0.5	–	−2.85	Mahy & Jacquemart (1999)
E. vulgare	Lab/captive	Comparison	Breeding design	10	0–0.5	–	−1.63	Melser *et al.* (1999)
S. latifolia	Lab/captive	Comparison	Breeding design	20	0–0.25	–	−1.14	Teixeira *et al.* (2008)

Studies that used pedigrees or breeding designs to estimate coefficients of inbreeding (*f*) or to create individuals of known *f* are shown without shading whereas studies that used other proxy methods to estimate inbreeding are shown in grey shading. 'Conditions' specifies whether a study was conducted in laboratory/captive conditions or in the wild. 'Design' specifies the use of two groups with distinct *f* values ('comparison'), or a range of individual *f* values ('regression'). 'Inbreeding estimation' refers to the method used to quantify inbreeding levels; estimation of *f* using pedigrees, homozygosity as a proxy (e.g. allozymes, microsatellites) or breeding designs to create individuals of known *f*. 'Sample size' is the number of individuals considered for a given study trait. 'Range of f' refers to the range of *f*, or to the values for inbred and outbred groups. For example, offspring produced by outbred full siblings will be $f = 0.25$. 'Reported effect' refers to the direction of the (significant) effect of inbreeding on the gametic trait as reported in the original article. 0 denotes an effect that was not significantly different from zero. '−B' is the inbreeding load (see main text for details). 'X' denotes data that could not be obtained. 'NA' denotes not applicable.
For illustrative purposes (see Fig. 2b), traits are classified into four categories as denoted by the symbols: *sperm quality or quantity trait, †sperm competition trait, ‡pollen quality or quantity trait and §pollen competition trait.

We calculated values of inbreeding load B, which provide a standard and comparable estimate of the change in the trait value with inbreeding, allowing quantitative comparisons across studies. From each original article, we extracted *f* and mean gametic trait values or requested them from the authors if the required data were not explicitly presented. We then calculated the inbreeding load (B) for each gametic trait measured in each study (hereafter 'study trait') as $B = \ln(\text{outbred-trait-mean}/\text{inbred-trait-mean})/f$ for experimental studies with discrete inbred and outbred groups, as minus the slope of a regression of ln(trait) on *f* for studies with a range of *f* values, or as $B = -\ln(1-\delta)/f$ for studies where the genetic load δ was provided (Charlesworth & Charlesworth, 1987; Keller & Waller, 2002). For ease of interpretation, we present −B rather than B so that negative values describe negative effects (Figs 1 and 2; Table 2).

Four animal studies (grey shading, Table 2) were excluded from quantitative analyses of B because they used molecular genetic proxies (typically microsatellite marker heterozygosity) to estimate inbreeding, which may not accurately predict *f* (Balloux *et al.*, 2004; Pemberton, 2004; Slate *et al.*, 2004), or used population-level rather than individual-level data. These four animal studies (11 study traits) are, however, retained in Table 2 for inspection of qualitative effects.

Considering all 183 study traits irrespective of the method used to estimate inbreeding (Table 2), the effect of inbreeding was reported (in the original paper) to be significantly negative for 115 study traits, not significantly different from zero for 66 study traits, significantly positive for one study trait (van Eldik *et al.*, 2006). The direction of the effect could not be obtained for one published study trait (Maximini *et al.*, 2011).

All 21 plant studies and 26 of the 30 animal studies (172 study traits in total) specified or estimated *f* using artificial breeding designs or natural pedigrees (black font, Table 2). Values of *f* ranged from 0 to 0.875 and

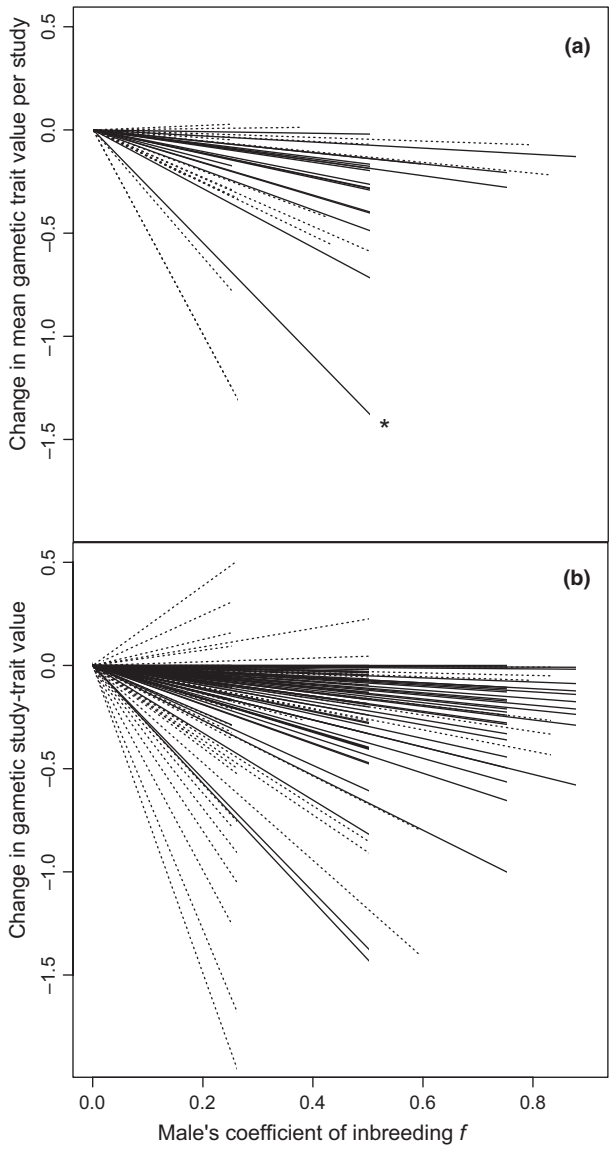

Fig. 1 Change in sperm (dotted lines) and pollen (solid lines) gametic trait values (log-transformed) in relation to the focal male's coefficient of inbreeding *f*. Each line represents (a) one study ($n = 35$) or (b) one study trait ($n = 126$), and each slope corresponds to (a) mean −B across the set of traits within each study and (b) −B for each study trait. Line lengths show the range of *f* considered in the published study. Outbred gametic trait values ($f = 0$) are centred to 0. For example, in the plant study marked with an asterisk (*), the mean difference in pollen traits between inbred ($f = 0.5$) and outbred individuals resulted in an inbreeding load (−B) equating to −1.43.

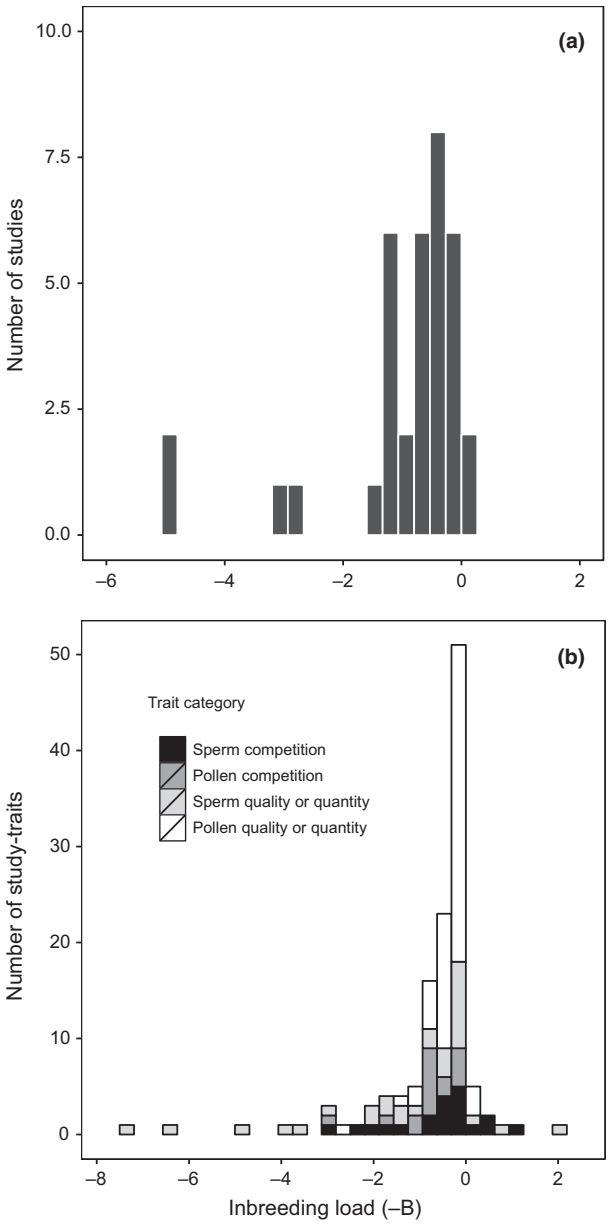

Fig. 2 Frequency distributions of inbreeding load (−B) across (a) studies and (b) study traits. In (b), gametic study traits are classified into four different categories: sperm quality/quantity traits, sperm competition traits, pollen quality/quantity traits and pollen competition traits.

inbreeding load (−B) ranged from −7.45 to 1.21, across 35 studies and 126 study traits (Figs 1 and 2, B and/or *f* could not be recovered for 46 study traits). The grand mean of −B across these studies was negative (mean ± SE: −0.98 ± 0.21, Figs 1a and 2a) and differed significantly from zero according to a one-sample *t*-test ($t_{34} = -4.76$, $P < 0.001$). We also used a mixed-effect model (nlme package in R software, R Core Team, 2013) to test whether estimated B differed between sperm and pollen study traits. Random study identity effects were fitted to account for nonindependence of B across multiple gametic traits considered in a single study. There was a nonsignificant tendency ($F_{1,33} = 3.27$, $P = 0.08$) for sperm study traits to show more negative values than pollen study traits (−B:

mean ± SE: −1.29 ± 0.40 and −0.58 ± 0.11, respectively), and both these effects differed from zero.

In studies that used more than one discrete inbred group, some study traits (37 in total) only differed in *f* (e.g. Good-Avila & Stephenson, 2003; see Table 2), therefore potentially creating some pseudo-replication. However, models fitted to data sets that comprised mean B across each set of study traits that differed only in *f* yielded similar results (not shown). The estimated magnitude of B did not vary with sample size across studies (Fig. S1). Furthermore, B did not increase significantly with maximum *f* (Fig. S2).

Discussion

Inbreeding depression in fitness-related traits is commonplace in normally outbreeding organisms (Charlesworth & Charlesworth, 1987; Keller & Waller, 2002; Charlesworth & Willis, 2009). However, as inbreeding depression is thought to result from homozygosity and therefore to be a property of diploid entities where genetic dominance effects are expressed, it is not immediately clear whether inbreeding depression in fitness components that stem from the performance of haploid gametes should be expected or what the magnitude could be. Although inbreeding depression in male fertilizing ability has been documented in some captive endangered species (Fitzpatrick & Evans, 2009; Roldan & Gomendio, 2009), there is no previous overview of the degree to which such effects span taxa, species, sperm and pollen traits or reproductive systems, even though the existence of substantial gametic inbreeding depression could affect sexual selection and mating system evolution and impact conservation management.

Together, the published studies summarized in our literature review showed a general negative effect of inbreeding on male gametic performance. The grand mean haploid inbreeding load reported across all studies (−B = −0.98) is within the range of estimated inbreeding loads in major fitness components such as survival or longevity (−B ranges from 0 to ca. −7.5 across multiple species, Keller & Waller, 2002; Laws & Jamieson, 2011) and in secondary sexual traits (Prokop *et al.*, 2010). For example, haploid inbreeding load in ornamental and courtship traits ranged from ca. −0.1 to −3.6 in guppies *Poecilia reticulata* (Van Oosterhout *et al.*, 2003). Inbreeding depression in male reproductive success could then be at least partially explained by inbreeding depression in male gametic performance occurring at the post-mating or post-pollination stage (i.e. 'post-copulatory' sexual selection).

However, our quantitative conclusions regarding the magnitude of inbreeding depression in male gametic performance should be interpreted with some caution. First, because more published studies reported significant inbreeding depression than did not, we cannot exclude the possibility of publication bias against non-significant results (Osenberg *et al.*, 1999; see also Supporting Information). Second, almost all published studies were conducted in laboratory or captive conditions. Although some studies used individuals collected or captured directly from wild populations, no study has quantified inbreeding depression in male gametic performance in free-living populations using an explicit measure of *f*. Effects occurring in wild populations may differ considerably from those estimated in laboratory and captive populations because captivity can substantially reduce inbreeding depression (Meagher *et al.*, 2000; Joron & Brakefield, 2003). The lack of estimates from wild populations probably reflects the challenge of simultaneously estimating both individual *f* and male gametic performance with adequate accuracy and precision. Populations with long-term pedigrees, and/or species with substantial genomic resources, and/or experimentally crossed plants further translocated into natural conditions offer the best opportunities to estimate inbreeding depression in aspects of male gametic performance in nature.

Inbreeding loads tended to be larger in sperm (i.e. animal) traits compared with pollen (i.e. plant) traits (−B = −1.29 and −0.58, respectively). Given such a difference, plants could potentially inbreed more than animals with less severe fitness consequences. Such differential inbreeding depression across kingdoms might be expected to stem from increased expression of haploid gametic genomes in plants vs. animals. The haploid pollen genome is known to be expressed from meiosis until the pollen tube enters the ovule and to facilitate pollen grain development, germination, growth and performance (Mascarenhas, 1993; Sari-Gorla & Frova, 1997; Stephenson *et al.*, 2001). There might therefore be less scope for inbreeding depression in pollen performance compared with sperm where the expression of haploid genes is generally thought to be more limited (Pizzari & Parker, 2009), and phenotype might consequently be more affected by diploid gene expression in males. However, recent studies suggest that haploid sperm genes might be expressed and drive sperm function (Joseph & Kirkpatrick, 2004; Immler, 2008; Pizzari & Foster, 2008). Overall, the relative influences of sperm and pollen haploid gene expression vs. diploid male gene expression on gamete development and subsequent performance remain poorly known and require further investigation. A generally smaller inbreeding load in pollen performance than in sperm performance might also be expected because most plant species included in our review can self-fertilize whereas the animal species cannot. Repeated self-fertilization leads to high levels of inbreeding, and therefore, purging of inbreeding load might be stronger and more effective in plants (Barrett & Charlesworth, 1991; Byers & Waller, 1999; Crnokrak & Barrett, 2002).

In general, the magnitude of inbreeding depression can be environment dependent and is often (but not always) greater in harsher environments (Armbruster & Reed, 2005; Waller *et al.*, 2008; Fox & Reed, 2011). Inbreeding might therefore be expected to affect male fertilization success to a greater degree in competitive contexts where gamete competition for fertilization success is high (Meagher *et al.*, 2000; Pizzari & Parker, 2009). Inbreeding could then substantially affect a male's fertilization success and fitness. We could not formally test whether estimated inbreeding loads in gametic performance were indeed greater under competitive vs. noncompetitive conditions because there are not yet sufficient studies that span both contexts. However, to qualitatively explore variation in estimated magnitudes of inbreeding depression among different types of traits, we classified gametic traits into four categories, comprising the quantity or quality of individual sperm or pollen, and the competitiveness of sperm or pollen in terms of fertilization success (Table 2, Fig. 2b). This categorization did not reveal any overwhelming differences in inbreeding depression between traits pertaining to gamete quantity or quality and traits pertaining to gamete competitiveness (Fig. 2b). However, in some species such as *Tribolium castaneum* (Michalczyk *et al.*, 2010), *Mesocricetus auratus* (Fritzsche *et al.*, 2006) and *Silene latifolia* (Austerlitz *et al.*, 2012), sperm or pollen traits related to gametic competition showed strong inbreeding depression in competitive contexts, but this was not accompanied by significant inbreeding depression in sperm quality or quantity traits measured independent of competitive outcomes (Table 2). This suggests that inbreeding, without (visibly) affecting gametic traits, can still decrease fertilization success in a competitive context. Future studies that measure both intrinsic gamete phenotype and competitiveness (i.e. fertilization success in a competitive context) are required to fully assess such effects and understand the implications for the evolution of mating systems including polyandry.

While our current review focussed on male gametic performance, female gametic performance might also show inbreeding depression, with further implications for evolution, population ecology and conservation. There is some evidence of inbreeding depression in the number of gametes produced by female plants (Carr & Dudash, 1995; Gargano *et al.*, 2011) and animals (Sewalem *et al.*, 1999; Janicke *et al.*, 2013). However, as female and male gametic traits are (by definition) different, future studies will need to consider different suites of traits in the context of female vs. male gametic inbreeding depression. Traits to be considered include physiological components of eggs and ovules, such as antioxidant, nutritive and hormonal constituents, which can greatly affect offspring growth and fitness (giving rise to maternal effects, Mousseau & Fox, 1998), and ovarian fluid, whose characteristics can influence female and male fertility (e.g. Gasparini & Pilastro, 2011).

Conclusion

Our review of published studies suggests that inbreeding can reduce male gametic performance, potentially reducing male reproductive success and hence fitness. Such inbreeding depression would not necessarily be expected given that gametes are haploid. However, due to the high diversity of gametic traits considered but low replication within traits, and the paucity of studies performed in the wild population, further studies are clearly required before the evolutionary or population dynamic consequences of inbreeding through male gametic performance can be fully understood. Future objectives should be to quantify inbreeding loads in wild populations, compare inbreeding depression in measures of intrinsic male gametic quantity or quality vs. fertilization success under competitive conditions, and quantify the relative roles of haploid vs. diploid gene expression on gamete performance.

Acknowledgments

We thank Łukasz Michalczyk, Louise D. Pedersen, Peter van Eldik, Stephen Robinson, Susan W. Margulis, María J. Carabaño, Giorgina Bernasconi, Arlee Montalvo, Thure P. Hauser and Andrew G. Stephenson for providing raw data or regression parameters associated with their published studies; Aurelio F. Malo, on behalf of the Chicago Zoological Society, for providing unpublished regression values; and Lukas Keller, Pirmin Nietlisbach, Matthew Wolak and Erik Postma for providing helpful comments and ideas. SL was supported by a Swiss National Science Foundation post-doctoral fellowship (PBBEP3_139396), an IEF Marie Curie fellowship and a Philip Leverhulme Prize awarded to JMR. JMR was supported by the UK Royal Society and the European Research Council. SMC was supported by the US National Science Foundation (award 0640881).

References

Agrawal, A.F. 2011. Are males the more 'sensitive' sex? *Heredity* **107**: 20–21.

Aitken, R.J. 1999. The amoroso lecture. The human spermatozoon – a cell in crisis? *J. Reprod. Fertil.* **115**: 1–7.

Aizen, M.A. & Harder, L.D. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* **88**: 271–281.

Aizen, M.A., Searcy, K.B. & Mulcahy, D.L. 1990. Among- and within-flower comparisons of pollen tube growth following self-and cross-pollinations in *Dianthus chinensis* (Caryophyllaceae). *Am. J. Bot.* **75**: 671–676.

Ala-Honkola, O., Uddström, A., Pauli, B.D. & Lindström, K. 2009. Strong inbreeding depression in male mating behaviour in a poeciliid fish. *J. Evol. Biol.* **22**: 1396–1406.

Ala-Honkola, O., Hosken, D.J., Manier, M.K., Lupold, S., Droge-Young, E.M., Berben, K.S. *et al.* 2013. Inbreeding

reveals mode of past selection on male reproductive characters in *Drosophila melanogaster*. *Ecol. Evol.* **3**: 2089–2102.
Alexander, N.J. & Anderson, D.J. 1987. Immunology of semen. *Fertil. Steril.* **47**: 192–205.
Allard, R.W. 1999. *Principles of Plant Breeding*. Wiley, New York, NY.
Ariizumi, T. & Toriyama, K. 2011. Genetic regulation of sporopollenin synthesis and pollen exine development. *Annu. Rev. Plant Biol.* **62**: 437–460.
Armbruster, P. & Reed, D.H. 2005. Inbreeding depression in benign and stressful environments. *Heredity* **95**: 235–242.
Asa, C., Miller, P., Agnew, M., Rebolledo, J.A.R., Lindsey, S.L., Callahan, M. *et al.* 2007. Relationship of inbreeding with sperm quality and reproductive success in Mexican gray wolves. *Anim. Conserv.* **10**: 326–331.
Ashman, T. 1998. Is relative pollen production or removal a good predictor of relative male fitness? An experimental exploration with a wild strawberry (*Fragaria virginiana*, Rosaceae). *Am. J. Bot.* **85**: 1166.
Aurich, C., Achmann, R. & Aurich, J.E. 2003. Semen parameters and level of microsatellite heterozygosity in Noriker draught horse stallions. *Theriogenology* **60**: 371–378.
Austerlitz, F., Gleiser, G., Teixeira, S. & Bernasconi, G. 2012. The effects of inbreeding, genetic dissimilarity and phenotype on male reproductive success in a dioecious plant. *Proc. R. Soc. B Biol. Sci.* **279**: 91–100.
Balloux, F., Amos, W. & Coulson, T. 2004. Does heterozygosity estimate inbreeding in real populations? *Mol. Ecol.* **13**: 3021–3031.
Barrett, S.C. 2002. The evolution of plant sexual diversity. *Nat. Rev. Genet.* **3**: 274–284.
Barrett, S. & Charlesworth, D. 1991. Effects of a change in the level of inbreeding on the genetic load. *Nature* **352**: 522–524.
Birkhead, T.R. 1998. Sperm competition in birds. *Rev. Reprod.* **3**: 123–129.
Birkhead, T.R. & Møller, A.P. 1998. *Sperm Competition and Sexual Selection*. Academic Press, San Diego, CA.
Birkhead, T.R., Martinez, J.G., Burke, T. & Froman, D.P. 1999. Sperm mobility determines the outcome of sperm competition in the domestic fowl. *Proc. R. Soc. B Biol. Sci.* **266**: 1759–1764.
Blackmore, S., Wortley, A.H., Skvarla, J.J. & Rowley, J.R. 2007. Pollen wall development in flowering plants. *New Phytol.* **174**: 483–498.
Blanco, J.M., Wildt, D.E., Höfle, U., Voelker, W. & Donoghue, A.M. 2009. Implementing artificial insemination as an effective tool for ex situ conservation of endangered avian species. *Theriogenology* **71**: 200–213.
Boorman, E. & Parker, G.A. 1976. Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol. Entomol.* **1**: 145–155.
Braun, R.E., Behringer, R.R., Peschon, J.J., Brinster, R.L. & Palmiter, R.D. 1989. Genetically haploid spermatids are phenotypically diploid. *Nature* **337**: 373–376.
Brekke, P., Bennett, P.M., Wang, J., Pettorelli, N. & Ewen, J.G. 2010. Sensitive males: inbreeding depression in an endangered bird. *Proc. R. Soc. B Biol. Sci.* **277**: 3677–3684.
Brown, J. & Caligari, P. 2013. *An Introduction to Plant Breeding*. Wiley-Blackwell, Oxford.
Burness, G., Casselman, S., Schulte-Hostedde, A., Moyes, C. & Montgomerie, R. 2004. Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). *Behav. Ecol. Sociobiol.* **56**: 65–70.
Byers, D. & Waller, D. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Syst.* **30**: 479–513.
Carr, D.E. & Dudash, M.R. 1995. Inbreeding depression under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. *Heredity* **75**: 437–445.
Casselman, S.J., Schulte-Hostedde, A.I. & Montgomerie, R. 2006. Sperm quality influences male fertilization success in walleye (*Sander vitreus*). *Can. J. Fish. Aquat. Sci.* **63**: 2119–2125.
Chang, C., Chen, Y.T., Yeh, S.D., Xu, Q., Wang, R.S., Guillou, F. *et al.* 2004. Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking the androgen receptor in Sertoli cells. *Proc. Natl. Acad. Sci. USA* **101**: 6876–6881.
Chapman, T. 2001. Seminal fluid-mediated fitness traits in *Drosophila*. *Heredity* **87**: 511–521.
Charlesworth, D. 2006. Evolution of plant breeding systems. *Curr. Biol.* **16**: 726–735.
Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**: 237–268.
Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression. *Genet. Res.* **74**: 329–340.
Charlesworth, D. & Willis, J.H. 2009. The genetics of inbreeding depression. *Nat. Rev. Genet.* **10**: 783–796.
Clutton-Brock, T.H. 1988. *Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems*. University of Chicago Press, Chicago, IL.
Cornwallis, C.K. & O'Connor, E.A. 2009. Sperm: seminal fluid interactions and the adjustment of sperm quality in relation to female attractiveness. *Proc. R. Soc. B Biol. Sci.* **276**: 3467–3475.
Cotton, S., Fowler, K. & Pomiankowski, A. 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. R. Soc. B Biol. Sci.* **271**: 771–783.
Crnokrak, P. & Barrett, S.C. 2002. Perspective: purging the genetic load: a review of the experimental evidence. *Evolution* **56**: 2347–2358.
Crnokrak, P. & Roff, D.A. 1999. Inbreeding depression in the wild. *Heredity* **83**: 260–270.
Cruzan, M.B. & Barrett, S.C. 1993. Contribution of cryptic incompatibility to the mating system of *Eichhornia paniculata* (Pontederiaceae). *Evolution* **47**: 925–934.
De Cauwer, I., Arnaud, J.F., Klein, E. & Dufay, M. 2012. Disentangling the causes of heterogeneity in male fecundity in gynodioecious *Beta vulgaris ssp. maritima*. *New Phytol.* **195**: 676–687.
De Gendt, K., Swinnen, J.V., Saunders, P.T.K., Schoonjans, L., Dewerchin, M., Devos, A. *et al.* 2004. A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. *Proc. Natl. Acad. Sci. USA* **101**: 1327–1332.
Denk, A.G., Holzmann, A., Peters, A., Vermeirssen, E.L.M. & Kempenaers, B. 2005. Paternity in mallards: effects of sperm quality and female sperm selection for inbreeding avoidance. *Behav. Ecol.* **16**: 825–833.
DeRose, M.A. & Roff, D.A. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution* **53**: 1288–1292.
Dickinson, H. & Elleman, C. 1985. Structural changes in the pollen grain of *Brassica oleracea* during dehydration in the

anther and development on the stigma as revealed by anhydrous fixation techniques. *Micron Microsc. Acta* **16**: 255–270.

Donoghue, A.M., Sonstegard, T.S., King, L.M., Smith, E.J. & Burt, D.W. 1999. Turkey sperm mobility influences paternity in the context of competitive fertilization. *Biol. Reprod.* **61**: 422–427.

Dou, X.Y., Yang, K.Z., Zhang, Y., Wang, W., Liu, X.L., Chen, L.Q. *et al.* 2011. WBC27, an adenosine tri-phosphate-binding cassette protein, controls pollen wall formation and patterning in *Arabidopsis*. *J. Integr. Plant Biol.* **53**: 74–88.

van Eldik, P., van der Waaij, E.H., Ducro, B., Kooper, A.W., Stout, T.A.E. & Colenbrander, B. 2006. Possible negative effects of inbreeding on semen quality in Shetland pony stallions. *Theriogenology* **65**: 1159–1170.

Enders, L. & Nunney, L. 2010. Sex-specific effects of inbreeding in wild-caught *Drosophila melanogaster* under benign and stressful conditions. *J. Evol. Biol.* **23**: 2309–2323.

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*. Longman, London.

Fitzpatrick, J.L. & Evans, J.P. 2009. Reduced heterozygosity impairs sperm quality in endangered mammals. *Biol. Lett.* **5**: 320–323.

Fox, C.W. & Reed, D.H. 2011. Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution* **65**: 246–258.

Fox, C.W., Xu, J., Wallin, W.G. & Curtis, C.L. 2012. Male inbreeding status affects female fitness in a seed-feeding beetle. *J. Evol. Biol.* **25**: 29–37.

de Franca, H., Hess, R.A. & de Franca, L.R. 2008. Spermatogenesis and cycle of the seminiferous epithelium. In: *Molecular Mechanisms in Spermatogenesis* (C.Y. Cheng, ed.), pp. 1–15. Landes Bioscience and Springer Science, Austin.

Fritzsche, P., Neumann, K., Nasdal, K. & Gattermann, R. 2006. Differences in reproductive success between laboratory and wild-derived golden hamsters *Mesocricetus auratus* as a consequence of inbreeding. *Behav. Ecol. Sociobiol.* **60**: 220–226.

Froman, D. 2003. Deduction of a model for sperm storage in the oviduct of the domestic fowl (*Gallus domesticus*). *Biol. Reprod.* **69**: 248–253.

Furness, C.A., Rudall, P.J. & Sampson, F.B. 2002. Evolution of microsporogenesis in angiosperms. *Int. J. Plant Sci.* **163**: 235–260.

Gage, M.J.G. & Morrow, E.H. 2003. Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. *Curr. Biol.* **13**: 754–757.

Gage, M.J.G., Macfarlane, C.P., Yeates, S., Ward, R.G., Searle, J.B. & Parker, G.A. 2004. Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. *Curr. Biol.* **14**: 44–47.

Gage, M.J.G., Surridge, A.K., Tomkins, J.L., Green, E., Wiskin, L., Bell, D.J. *et al.* 2006. Reduced heterozygosity depresses sperm quality in wild rabbits, *Oryctolagus cuniculus*. *Curr. Biol.* **16**: 612–617.

Gargano, D., Gullo, T. & Bernardo, L. 2011. Fitness drivers in the threatened *Dianthus guliae Janka* (Caryophyllaceae): disentangling effects of growth context, maternal influence and inbreeding depression. *Plant Biol.* **13**: 96–103.

Gasparini, C. & Pilastro, A. 2011. Cryptic female preference for genetically unrelated males is mediated by ovarian fluid in the guppy. *Proc. R. Soc. B Biol. Sci.* **278**: 2495–2501.

Gasparini, C., Simmons, L.W., Beveridge, M. & Evans, J.P. 2010. Sperm swimming velocity predicts competitive fertilization success in the green swordtail *Xiphophorus helleri*. *PLoS ONE* **5**: e12146.

Gillott, C. 1996. Male insect accessory glands: functions and control of secretory activity. *Invertebr. Reprod. Dev.* **30**: 199–205.

Gomendio, M. & Roldan, E. 2008. Implications of diversity in sperm size and function for sperm competition and fertility. *Int. J. Dev. Biol.* **52**: 439–447.

Gomendio, M., Cassinello, J. & Roldan, E.R.S. 2000. A comparative study of ejaculate traits in three endangered ungulates with different levels of inbreeding: fluctuating asymmetry as an indicator of reproductive and genetic stress. *Proc. R. Soc. B Biol. Sci.* **267**: 875–882.

Good-Avila, S.V. & Stephenson, A.G. 2003. Parental effects in a partially self-incompatible herb *Campanula rapunculoides* L. (Campanulaceae): influence of variation in the strength of self-incompatibility on seed set and progeny performance. *Am. Nat.* **161**: 615–630.

Goodwillie, C., Kalisz, S. & Eckert, C.G. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.* **36**: 47–79.

Guo, J.X. & Liu, Y.G. 2012. Molecular control of male reproductive development and pollen fertility in riceF. *J. Integr. Plant Biol.* **54**: 967–978.

Hauser, T.P. & Siegismund, H. 2000. Inbreeding and outbreeding effects on pollen fitness and zygote survival in *Silene nutans* (Caryophyllaceae). *J. Evol. Biol.* **13**: 446–454.

Hayes, C.N., Winsor, J.A. & Stephenson, A.G. 2004. Inbreeding influences herbivory in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *Oecologia* **140**: 601–608.

Hayes, C.N., Winsor, J.A. & Stephenson, A.G. 2005a. A comparison of male and female responses to inbreeding in *Cucurbita pepo* subsp. *texana* (Cucurbitaceae). *Am. J. Bot.* **92**: 107–115.

Hayes, C.N., Winsor, J.A. & Stephenson, A.G. 2005b. Multigenerational effects of inbreeding in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *Evolution* **59**: 276–286.

Hedrick, P.W. & Kalinowski, S.T. 2000. Inbreeding depression in conservation biology. *Annu. Rev. Ecol. Syst.* **31**: 139–162.

Higginson, D.M. & Pitnick, S. 2011. Evolution of intra-ejaculate sperm interactions: do sperm cooperate? *Biol. Rev.* **86**: 249–270.

Holsinger, K.E. & Thomson, J.D. 1994. Pollen discounting in *Erythronium grandiflorum*: mass-action estimates from pollen transfer dynamics. *Am. Nat.* **144**: 799–812.

Hughes, K.A. 1997. Quantitative genetics of sperm precedence in *Drosophila melanogaster*. *Genetics* **145**: 139–151.

Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N. & Vellend, M. 2008. Ecological consequences of genetic diversity. *Ecol. Lett.* **11**: 609–623.

Humphries, S., Evans, J. & Simmons, L. 2008. Sperm competition: linking form to function. *BMC Evol. Biol.* **8**: 319.

Hunter, F.M. & Birkhead, T.R. 2002. Sperm viability and sperm competition in insects. *Curr. Biol.* **12**: 121–123.

Husband, B.C. & Schemske, D.W. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54–70.

Immler, S. 2008. Sperm competition and sperm cooperation: the potential role of diploid and haploid expression. *Reproduction* **135**: 275–283.

Immler, S., Saint-Jalme, M., Lesobre, L., Sorci, G., Roman, Y. & Birkhead, T.R. 2007. The evolution of sperm morphometry in pheasants. *J. Evol. Biol.* **20**: 1008–1014.

Janicke, T., Vellnow, N., Sarda, V. & David, P. 2013. Sex-specific inbreeding depression depends on the strength of male–male competition. *Evolution* **67**: 2861–2875.

Jennions, M.D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**: 21–64.

Jóhannsson, M., Gates, M. & Stephenson, A. 1998. Inbreeding depression affects pollen performance in *Cucurbita texana*. *J. Evol. Biol.* **11**: 579–588.

Joron, M. & Brakefield, P.M. 2003. Captivity masks inbreeding effects on male mating success in butterflies. *Nature* **424**: 191–194.

Joseph, S.B. & Kirkpatrick, M. 2004. Haploid selection in animals. *Trends Ecol. Evol.* **19**: 592–597.

Keller, L.F. & Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**: 230–241.

Knobil, E. & Neil, J.D. 1994. *The Physiology of Reproduction*. Raven Press, New York, NY.

Konior, M., Keller, L. & Radwan, J. 2005. Effect of inbreeding and heritability of sperm competition success in the bulb mite *Rhizoglyphus robini*. *Heredity* **94**: 577–581.

Lande, R. & Schemske, D.W. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**: 24–40.

Lankinen, Å. & Armbruster, W.S. 2007. Pollen competition reduces inbreeding depression in *Collinsia heterophylla* (Plantaginaceae). *J. Evol. Biol.* **20**: 737–749.

Laws, R.J. & Jamieson, I.G. 2011. Is lack of evidence of inbreeding depression in a threatened New Zealand robin indicative of reduced genetic load? *Anim. Conserv.* **14**: 47–55.

Levitan, D.R. 2000. Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. *Proc. R. Soc. B Biol. Sci.* **267**: 531–534.

Li, N., Zhang, D.S., Liu, H.S., Yin, C.S., Li, X.X., Liang, W.Q. *et al.* 2006. The rice tapetum degeneration retardation gene is required for tapetum degradation and anther development. *Plant Cell* **18**: 2999–3014.

Li, H., Yuan, Z., Vizcay-Barrena, G., Yang, C., Liang, W., Zong, J. *et al.* 2011. Persistent tapetal cell1 encodes a PHD-finger protein that is required for tapetal cell death and pollen development in rice. *Plant Physiol.* **156**: 615–630.

Liu, L. & Fan, X.D. 2013. Tapetum: regulation and role in sporopollenin biosynthesis in Arabidopsis. *Plant Mol. Biol.* **83**: 165–175.

Losdat, S., Richner, H., Blount, J.D. & Helfenstein, F. 2011. Immune activation reduces sperm quality in the great tit. *PLoS ONE* **6**: e22221.

Lüpold, S., Linz, G.M. & Birkhead, T.R. 2009. Sperm design and variation in the New World blackbirds (Icteridae). *Behav. Ecol. Sociobiol.* **63**: 899–909.

Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland.

Mahy, G. & Jacquemart, A.L. 1999. Early inbreeding depression and pollen competition in *Calluna vulgaris* (L.) *Hull*. *Ann. Bot.* **83**: 697–704.

Mallet, M.A. & Chippindale, A.K. 2011. Inbreeding reveals stronger net selection on *Drosophila melanogaster* males: implications for mutation load and the fitness of sexual females. *Heredity* **106**: 994–1002.

Malo, A.F., Garde, J.J., Soler, A.J., Garcia, A.J., Gomendio, M. & Roldan, E.R.S. 2005. Male fertility in natural populations of red deer is determined by sperm velocity and the proportion of normal spermatozoa. *Biol. Reprod.* **72**: 822–829.

Malo, A.F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A.J. & Roldan, E.R.S. 2006. Sperm design and sperm function. *Biol. Lett.* **2**: 246–249.

Malo, A.F., Martinez-Pastor, F., Alaks, G., Dubach, J. & Lacy, R.C. 2010. Effects of genetic captive-breeding protocols on sperm quality and fertility in the white-footed mouse. *Biol. Reprod.* **83**: 540–548.

Margulis, S.W. & Walsh, A. 2002. The effects of inbreeding on testicular sperm concentration in *Peromyscus polionotus*. *Reprod. Fertil. Dev.* **14**: 63–67.

Mariette, M., Kelley, J.L., Brooks, R. & Evans, J.P. 2006. The effects of inbreeding on male courtship behaviour and coloration in guppies. *Ethology* **112**: 807–814.

Martin, P.A., Reimers, T.J., Lodge, J.R. & Dziuk, P.J. 1974. The effect of ratios and numbers of spermatozoa mixed from two males on proportions of offspring. *J. Reprod. Fertil.* **39**: 251–258.

Mascarenhas, J.P. 1993. Molecular mechanisms of pollen tube growth and differentiation. *Plant Cell* **5**: 1303.

Maximini, L., Fuerst-Waltl, B., Gredler, B. & Baumung, R. 2011. Inbreeding depression on semen quality in Austrian dual-purpose Simmental bulls. *Reprod. Domest. Anim.* **46**: e102–e104.

Meagher, S., Penn, D.J. & Potts, W.K. 2000. Male-male competition magnifies inbreeding depression in wild house mice. *Proc. Natl. Acad. Sci. USA* **97**: 3324–3329.

Mehlis, M., Frommen, J.G., Rahn, A.K. & Bakker, T. 2012. Inbreeding in three-spined sticklebacks (*Gasterosteus aculeatus* L.): effects on testis and sperm traits. *Biol. J. Linn. Soc.* **107**: 510–520.

Melser, C., Bijleveld, A. & Klinkhamer, P.G. 1999. Late-acting inbreeding depression in both male and female function of *Echium vulgare* (Boraginaceae). *Heredity* **83**: 162–170.

Michalczyk, Å., Martin, O.Y., Millard, A.L., Emerson, B.C. & Gage, M.J.G. 2010. Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male *Tribolium castaneum*. *Proc. R. Soc. B Biol. Sci.* **277**: 3483–3491.

Montalvo, A.M. 1992. Relative success of self and outcross pollen comparing mixed-and single-donor pollinations in *Aquilegia caerulea*. *Evolution* **46**: 1181–1198.

Mousseau, T.A. & Fox, C.W. 1998. *Maternal Effects as Adaptations*. Oxford University Press, New York, NY.

Muchhala, N. & Thomson, J.D. 2010. Fur versus feathers: pollen delivery by bats and hummingbirds and consequences for pollen production. *Am. Nat.* **175**: 717–726.

Mulcahy, D.L. & Mulcahy, G.B. 1987. The effects of pollen competition. *Am. Sci.* **75**: 44–50.

Nelson Hayes, C., Winsor, J.A. & Stephenson, A.G. 2005. Environmental variation influences the magnitude of inbreeding depression in *Cucurbita pepo* ssp. *texana* (Cucurbitaceae). *J. Evol. Biol.* **18**: 147–155.

Okada, K., Blount, J.D., Sharma, M.D., Snook, R.R. & Hosken, D.J. 2011. Male attractiveness, fertility and susceptibility to oxidative stress are influenced by inbreeding in *Drosophila simulans*. *J. Evol. Biol.* **24**: 363–371.

Osenberg, C.W., Sarnelle, O., Cooper, S.D. & Holt, R.D. 1999. Resolving ecological questions through meta-analysis: goals, metrics, and models. *Ecology* **80**: 1105–1117.

Parish, R.W. & Li, S.F. 2010. Death of a tapetum: a programme of developmental altruism. *Plant Sci.* **178**: 73–89.

Parker, G.A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**: 525–567.

Parker, G.A. 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *J. Theor. Biol.* **96**: 281–294.

Parker, G.A. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In: *Sperm Competition and Sexual Selection* (T.R. Birkhead & A.P. Møller, eds), pp. 3–54. Academic Press, London.

Parker, G.A. & Begon, M.E. 1993. Sperm competition games: sperm size and number under gametic control. *Proc. R. Soc. B Biol. Sci.* **253**: 255–262.

Pasonen, H.-L., Pulkkinen, P. & Käpylä, M. 2001. Do pollen donors with fastest-growing pollen tubes sire the best offspring in an anemophilous tree, *Betula pendula* (Betulaceae)? *Am. J. Bot.* **88**: 854–860.

Pedersen, L.D., Pedersen, A.R., Bijlsma, R. & Bundgaard, J. 2011. The effects of inbreeding and heat stress on male sterility in *Drosophila melanogaster*. *Biol. J. Linn. Soc.* **104**: 432–442.

Pemberton, J. 2004. Measuring inbreeding depression in the wild: the old ways are the best. *Trends Ecol. Evol.* **19**: 613–615.

Perry, L.E. & Dorken, M.E. 2011. The evolution of males: support for predictions from sex allocation theory using mating arrays of *Sagittaria latifolia* (Alismataceae). *Evolution* **65**: 2782–2791.

Petrovic, V.C., Maksimovic, N., Petrovic, M.P., Petrovic, M.M., Ilic, Z.Z., Muslic, D.R. *et al.* 2013. Effect of inbreeding on body growth traits and sperm DNA fragmentation level in rams. *Anim. Sci. Pap. Rep.* **31**: 27–33.

Piffanelli, P., Ross, J.H. & Murphy, D. 1998. Biogenesis and function of the lipidic structures of pollen grains. *Sex. Plant Reprod.* **11**: 65–80.

Pike, T.W., Blount, J.D., Lindström, J. & Metcalfe, N.B. 2010. Dietary carotenoid availability, sexual signalling and functional fertility in sticklebacks. *Biol. Lett.* **6**: 191–193.

Pizzari, T. & Birkhead, T.R. 2000. Female feral fowl eject sperm of subdominant males. *Nature* **405**: 787–789.

Pizzari, T. & Foster, K.R. 2008. Sperm sociality: cooperation, altruism, and spite. *PLoS Biol.* **6**: e130.

Pizzari, T. & Parker, G.A. 2009. Sperm competition and sperm phenotype. In: *Sperm Biology: An Evolutionary Perspective* (T.R. Birkhead, D.J. Hosken & S. Pitnick, eds), pp. 207–245. Academic Press, San Diego, CA.

Pizzari, T., Lø, H. & Cornwallis, C.K. 2004. Sex-specific, counteracting responses to inbreeding in a bird. *Proc. R. Soc. B Biol. Sci.* **271**: 2115–2121.

Pizzari, T., Worley, K., Burke, T. & Froman, D. 2008. Sperm competition dynamics: ejaculate fertilising efficiency changes differentially with time. *BMC Evol. Biol.* **8**: 332.

Pölkki, M., Krams, I., Kangassalo, K. & Rantala, M.J. 2012. Inbreeding affects sexual signalling in males but not females of *Tenebrio molitor*. *Biol. Lett.* **8**: 423–425.

Prokop, Z., Leś, J., Banaś, P., Koteja, P. & Radwan, J. 2010. Low inbreeding depression in a sexual trait in the stalk-eyed fly *Teleopsis dalmanni*. *Evol. Ecol.* **24**: 827–837.

R Core Team 2013. *R: A Language and Environment for Statistical Computing*. R Core Team, Vienna, Austria.

Rausher, M.D. & Chang, S.M. 1999. Stabilization of mixed-mating systems by differences in the magnitude of inbreeding depression for male and female fitness components. *Am. Nat.* **154**: 242–248.

Reed, D.H. & Frankham, R. 2003. Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**: 230–237.

Reid, J., Arcese, P., Cassidy, A.E., Marr, A., Smith, J.M. & Keller, L. 2005. Hamilton and Zuk meet heterozygosity? Song repertoire size indicates inbreeding and immunity in song sparrows (*Melospiza melodia*). *Proc. R. Soc. B Biol. Sci.* **272**: 481–487.

Robertson, K.M., O'Donnell, L., Jones, M.E.E., Meachem, S.J., Boon, W.C., Fisher, C.R. *et al.* 1999. Impairment of spermatogenesis in mice lacking a functional aromatase (cyp 19) gene. *Proc. Natl. Acad. Sci. USA* **96**: 7986–7991.

Robinson, S.P., Kennington, W.J. & Simmons, L.W. 2009. No evidence for optimal fitness at intermediate levels of inbreeding in *Drosophila melanogaster*. *Biol. J. Linn. Soc.* **98**: 501–510.

Roff, D.A. 2002. Inbreeding depression: tests of the overdominance and partial dominance hypotheses. *Evolution* **56**: 768–775.

Roldan, E.R.S. & Gomendio, M. 2009. Sperm and conservation. In: *Sperm Biology: An Evolutionary Perspective* (T.R. Birkhead, D.J. Hosken & S. Pitnick, eds), pp. 539–555. Academic Press, San Diego, CA.

Roldan, E.R.S., Cassinello, J., Abaigar, T. & Gomendio, M. 1998. Inbreeding, fluctuating asymmetry, and ejaculate quality in an endangered ungulate. *Proc. R. Soc. B Biol. Sci.* **265**: 243–248.

Roosen-Runge, E.C. 1962. The process of spermatogenesis in mammals. *Biol. Rev.* **37**: 343–376.

Ruiz-Lòpez, M.J., Evenson, D.P., Espeso, G., Gomendio, M. & Roldan, E.R.S. 2010. High levels of DNA fragmentation in spermatozoa are associated with inbreeding and poor sperm quality in endangered ungulates. *Biol. Reprod.* **83**: 332–338.

Ruiz-Lòpez, M.J., Gañan, N., Godoy, J.A., Del Olmo, A., Garde, J., Espeso, G. *et al.* 2012. Heterozygosity-fitness correlations and inbreeding depression in two critically endangered mammals. *Conserv. Biol.* **26**: 1121–1129.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**: 491–494.

Sanz, L., Calvete, J.J., Mann, K., Gabius, H.-J. & Töpfer-Petersen, E. 1993. Isolation and biochemical characterization of heparin-binding proteins from boar seminal plasma: a dual role for spermadhesins in fertilization. *Mol. Reprod. Dev.* **35**: 37–43.

Sari-Gorla, M. & Frova, C. 1997. *Pollen Tube Growth and Pollen Selection Improvement*. Cambridge University Press, New York, NY.

Schemske, D.W. & Lande, R. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical Observations. *Evolution* **39**: 41–52.

Scott, R.J., Spielman, M. & Dickinson, H. 2004. Stamen structure and function. *Plant Cell* **16**: S46–S60.

Sewalem, A., Johansson, K., Wilhelmson, M. & Lillpers, K. 1999. Inbreeding and inbreeding depression on reproduction and production traits of White Leghorn lines selected for egg production traits. *Br. Poult. Sci.* **40**: 203–208.

Shivanna, K.R. & Johri, B.M. 1985. *The Angiosperm Pollen: Structure and Function*. Wiley Eastern, New Delhi.

Sies, H. 1991. *Oxidative Stress: Oxidants and Antioxidants*. London Academic Press, London.

Simmons, L.W. 2005. The evolution of polyandry: sperm competition, sperm selection, and offspring viability. *Annu. Rev. Ecol. Evol. Syst.* **36**: 125–146.

Simmons, L.W. 2011. Inbreeding depression in the competitive fertilization success of male crickets. *J. Evol. Biol.* **24**: 415–421.

Simmons, L.W. & Moore, A.J. 2009. Evolutionary quantitative genetics of sperm. In: *Sperm Biology: An Evolutionary Perspective* (T.R. Birkhead, D.J. Hosken & S. Pitnick, eds), pp. 406–427. Academic Press, San Diego, CA.

Slate, J., David, P., Dodds, K.G., Veenvliet, B.A., Glass, B.C., Broad, T.E. *et al.* 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* **93**: 255–265.

Snook, R.R. 2005. Sperm in competition: not playing by the numbers. *Trends Ecol. Evol.* **20**: 46–53.

Snow, A.A. & Spira, T.P. 1991. Pollen vigour and the potential for sexual selection in plants. *Nature* **352**: 796–797.

Snow, A.A. & Spira, T.P. 1996. Pollen-tube competition and male fitness in *Hibiscus moscheutos*. *Evolution* **50**: 1866–1870.

Stephenson, A., Hayes, C., Johannsson, M. & Winsor, J. 2001. The performance of microgametophytes is affected by inbreeding depression and hybrid vigor in the sporophytic generation. *Sex. Plant Reprod.* **14**: 77–83.

Stephenson, A.G., Leyshon, B., Travers, S.E., Hayes, C.N. & Winsor, J.A. 2004. Interrelationships among inbreeding, herbivory, and disease on reproduction in a wild gourd. *Ecology* **85**: 3023–3034.

Stockley, P. 1999. Sperm selection and genetic incompatibility: does relatedness of mates affect male success in sperm competition? *Proc. R. Soc. B Biol. Sci.* **266**: 1663–1669.

Szulkin, M., Garant, D., McCleery, R.H. & Sheldon, B.C. 2007. Inbreeding depression along a life-history continuum in the great tit. *J. Evol. Biol.* **20**: 1531–1543.

Teixeira, S., Foerster, K. & Bernasconi, G. 2008. Evidence for inbreeding depression and post-pollination selection against inbreeding in the dioecious plant *Silene latifolia*. *Heredity* **102**: 101–112.

Till-Bottraud, I., Joly, D., Lachaise, D. & Snook, R. 2005. Pollen and sperm heteromorphism: convergence across kingdoms? *J. Evol. Biol.* **18**: 1–18.

Tregenza, T. & Wedell, N. 2000. Genetic compatibility, mate choice and patterns of parentage: invited review. *Mol. Ecol.* **9**: 1013–1027.

Tremellen, K. 2008. Oxidative stress and male infertility – a clinical perspective. *Hum. Reprod. Update* **14**: 243–258.

Twell, D. 2011. Male gametogenesis and germline specification in flowering plants. *Sex. Plant Reprod.* **24**: 149–160.

Van Oosterhout, C., Trigg, R.E., Carvalho, G.R., Magurran, A.E., Hauser, L. & Shaw, P.W. 2003. Inbreeding depression and genetic load of sexually selected traits: how the guppy lost its spots. *J. Evol. Biol.* **16**: 273–281.

Walker, W.H. 2011. Testosterone signaling and the regulation of spermatogenesis. *Spermatogenesis* **1**: 116–120.

Waller, D.M., Dole, J. & Bersch, A.J. 2008. Effects of stress and phenotypic variation on inbreeding depression in *Brassica rapa*. *Evolution* **62**: 917–931.

Wang, J., Fan, H.C., Behr, B. & Quake Stephen, R. 2012. Genome-wide single-cell analysis of recombination activity and de novo mutation rates in human sperm. *Cell* **150**: 402–412.

Weller, S.G. & Ornduff, R. 1991. Pollen tube growth and inbreeding depression in *Amsinckia grandiflora* (Boraginaceae). *Am. J. Bot.* **78**: 801–804.

Wells, H. 1979. Self-fertilization: advantageous or deleterious? *Evolution* **33**: 252–255.

White-Cooper, H., Doggett, K. & Ellis, R.E. 2009. The evolution of spermatogenesis. In: *Sperm Biology: An Evolutionary Perspective* (T.R. Birkhead, D.J. Hosken & S. Pitnick, eds), pp. 152–185. Academic Press, San Diego, CA.

Whitlock, M.C. & Agrawal, A.F. 2009. Purging the genome with sexual selection: reducing mutation load through selection on males. *Evolution* **63**: 569–582.

Wigby, S., Sirot, L.K., Linklater, J.R., Buehner, N., Calboli, F.C.F., Bretman, A. *et al.* 2009. Seminal fluid protein allocation and male reproductive success. *Curr. Biol.* **19**: 751–757.

Wildt, D.E., Baas, E.J., Chakraborty, P.K., Wolfle, T.L. & Stewart, A.P. 1982. Influence of inbreeding on reproductive performance, ejaculate quality and testicular volume in the dog. *Theriogenology* **17**: 445–452.

Wildt, D.E., Bush, M., Goodrowe, K.L., Packer, C., Pusey, A.E., Brown, J.L. *et al.* 1987. Reproductive and genetic consequences of founding isolated lion populations. *Nature* **329**: 328–331.

Wildt, D.E., Pukazhenthi, B., Brown, J.L., Monfort, S., Howard, J. & Roth, T. 1995. Spermatology for understanding, managing and conserving rare species. *Reprod. Fertil. Dev.* **7**: 811–824.

Willing, R., Bashe, D. & Mascarenhas, J. 1988. An analysis of the quantity and diversity of messenger RNAs from pollen and shoots of Zea mays. *Theor. Appl. Genet.* **75**: 751–753.

Wolfner, M.F. 2002. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in Drosophila. *Heredity* **88**: 85–93.

Yacine, A. & Bouras, F. 1997. Self- and cross-pollination effects on pollen tube growth and seed set in holm oak *Quercus ilex* L (Fagaceae). *Ann. Sci. Forest.* **54**: 447–462.

Zajitschek, S.R.K. & Brooks, R.C. 2010. Inbreeding depression in male traits and preference for outbred males in *Poecilia reticulata*. *Behav. Ecol.* **21**: 884–891.

Zajitschek, S.R.K., Lindholm, A.K., Evans, J.P. & Brooks, R.C. 2009. Experimental evidence that high levels of inbreeding depress sperm competitiveness. *J. Evol. Biol.* **22**: 1338–1345.

Zeh, J.A. & Zeh, D.W. 2001. Reproductive mode and the genetic benefits of polyandry. *Anim. Behav.* **61**: 1051–1063.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Relationship between sample size (N) and mean inbreeding load ($-B$) across all studies.

Figure S2 Relationship between inbreeding load ($-B$) and the coefficient of inbreeding (f).

Received 7 January 2014; revised 1 April 2014; accepted 2 April 2014