An unrecognized genetic predisposition for sperm competition in aphids

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Abstract

A hitherto unrecognized genetic predisposition for sperm competition exists in aphids. The females of a clone are diploid for the autosomes and sex chromosomes: AA/XX (where A stands for a haploid set of autosomes). Production of males is parthenogenetic, except for the random loss of one X chromosome. Male offspring is diploid for the autosomes but haploid for the sex chromosomes (AA/X0). Hence, each clone produces two brotherhoods of males. The relatedness of males sharing the same X chromosome will be 1. Their relatedness to males of the same clone that received the other X chromosome will be less than 1. Sperm results from meiosis and is haploid, but the sperm cells that do not receive an X chromosome die. The surviving sperm cells share the male's X chromosome, which makes up the largest part of the genome. Thus, the two brotherhoods of males of a clone produce two kinships of sperm. Sperm cells carrying the same X chromosome will share a part of the genome that is larger than 0.5. Those carrying different X chromosomes will share a part less than 0.5.

The above can lead to sperm competition between males from the same clone. Competition from males of different clones can increase sperm competition even further. Ecological factors mixing different clones will have this effect. Sperm cooperation within ejaculates is a possible adaptation to sperm competition. Cystic spermatogenesis, in turn, is a physiological preadaptation for sperm cooperation. Sperm cooperation should thus be as frequent in aphids as in related taxa, like scale insects. Yet, the evidence is inconclusive. This discrepancy between theory and evidence demands an explanation.

Key words: sperm competition, sperm cooperation, sperm relatedness, Aphidoidea

1. Aphid sex determination

Sex determination is chromosomal in aphids. Animals with two X chromosomes are female (XX), those with only one are male (X0). Aphids reproduce through cyclic parthenogenesis. Parthenogenetic females produce clonal offspring throughout the season, sexual females and males produce recombinant offspring at its end. The recombinant eggs overwinter, and the hatching animals reproduce through parthenogenesis, again.

Sex determination happens during parthenogenetic egg production in the mothers of the sexual animals. Female offspring results from an egg receiving two X chromosomes, male offspring results from an egg receiving only one. The other gets lost (Spence and Blackman 1998). Both X chromosomes usually get lost at an equal risk during the production of males (Wilson, Sunnucks, and Hales 1997; Singh and Singh 2022, 271). Male offspring is otherwise clonal to their mothers. That is, males are diploid for the autosomes but haploid for the X chromosome (AA/X0). They produce haploid sperm that will only survive if it receives the X chromosome. Sperm that receive no X chromosome degenerates. Recombinant eggs thus receive two X chromosomes, again, and become (parthenogenetic) females.

2. Relatedness of sperm from one aphid clone

Ignoring new mutations, the females of an aphid clone are genetically identical. This includes parthenogenetic and sexual females. Since males receive only one of the two X chromosomes of their mothers/clone (hereinafter either X_1 or X_2), each clone produces two brotherhoods of males (carrying X_10 or X_20). Since sperm cells lacking an X chromosome die, these brotherhoods produce sperm of two kinships (carrying X_1 or X_2).[1]

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[1] Autosome behaviour during spermatogenesis can differ between aphids: crossing-over recombination between homologues does not occur in every species (Manicardi, Mandrioli, and Blackman 2015). I ignore this intricacy for simplicity.

Most aphid species have a small number of chromosomes (4-10 pairs is common), and the X chromosomes are the largest (Blackman 1980). The following will consider the proportion, *p*, of a clone's genome carried by males and sperm of that clone.

Suppose a clone is heterozygous for its X chromosomes. One half of its males will share one of its X chromosomes, and the remaining half will share the other. The two X chromosomes take the largest part of a clone's genome. According to Wilson et al. (1997, p. 235), it amounts to 28% in an Australian descendant of the Holarctic *Sitobion fragariae* (Walker). Since males lack one X chromosome (14%), they carry only 0.86 of their clone's genome. Those males of a clone that receive different X chromosomes will only share the autosomal part, p = 0.72. Although producing males involves no meiosis, they can differ in their X chromosomes (fig. 1, male AA/X0 genomes).

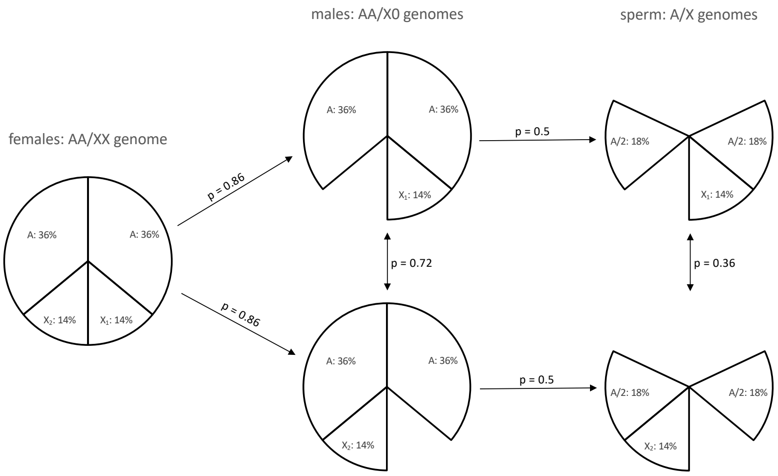

Figure 1: Proportion, *p*, of the diploid clone's genome in Australian *S. fragariae*.[3] Two X chromosomes of diploid females constitute 28% of their AA/XX genome. Males get a diploid set of autosomes but lack one X chromosome. Hence, their AA/X0 genomes carry 0.86 of the diploid AA/XX genomes. Meiosis during spermiogenesis reduces the autosomal part of the male genomes to yield a haploid A/X genome. While this is 0.5 of the clone's genome, spermatozoa with different X chromosomes share less than 0.5 of the clone's genome with each other (0.36).

Sperm cells are haploid through meiosis, but those that do not get an X chromosome degenerate. Surviving sperm cells of a male share half of their autosomal genome, but the whole of their X chromosome. A balanced cell cycle requires sperm cells to be haploid, to form diploid zygotes by fusing with eggs. Yet, sperm cells carry a larger part of the male's genome (AA/X0). Furthermore, males of the same clone that carry different X chromosomes share only 0.72 of the clone's genome. Hence, sperm cells of the same clone with different X chromosomes share only 0.36 of the clone's genome (fig. 1, sperm A/X-genomes). Sperm from males of the same clone can still differ in their kinship.

This results in a genetic predisposition for sperm competition. The lack of adaptations to sperm competition in aphid species surprises in consequence. The widespread existence of adaptations to sperm competition in species without the genetic predisposition of aphids intensifies this surprise. The kinship between the sperm cells of a male with normal meiosis will be 0.5, but lower between unrelated males. Competition between sperm of unrelated males has resulted in various adaptations (see: Parker 1970; Eberhard 1985; Trivers 1985, 121; H. Moore et al. 2002; Swallow and Wilkinson 2002; Pitnick, Hosken, and Birkhead 2009). Cooperation of sperm cells from the same male is one form of adaptation to sperm competition. Selection at the organism level can produce this cooperation at the cell level (Higginson and Pitnick 2011).

3. Further factors facilitating sperm competition

First, genes that get expressed in males have a bias to accumulate on the X chromosome in aphids (e.g., Jaquiéry et al. 2013; Jaquiéry et al. 2022; Li, Bickel, et al. 2020; Li, Zhang, et al. 2020).

Second, cystic spermatogenesis is a preadaptation for transferring sperm in bundles. Daughter cells of stem cells (*spermatogonia*) divide mitotically a species-specific number of times. Then meiosis produces haploid cells (*spermatids*) and their differentiation (*spermiogenesis*) results in mature sperm. Nourishing cells meanwhile form a cap (cyst) enclosing each growing bundle of gamete-cells. Sperm transfer will only be individual when the cyst cells degenerate before transfer (see Supplement A). If each spermatid receives one of two sex chromosomes and matures, the cells in a cyst will share 0.5 of their genes. In aphids, however, the spermatids that receive no X chromosome die leaving a bundle of sperm cells that are clonal for their X chromosome.

Third, various ecological factors and life history traits can mix aphid clones. This will enhance the likelihood that males from different clones compete. For example: aphid population density, colony forming tendency of individuals, host plant distribution, density dependent induction of winged morphs, laying of recombinant eggs in clutches, programmed occurrence of winged morphs and migration, programmed host alternation (see Supplement B).

4. Evidence on sperm-bundles in aphids

The evidence on sperm bundles in aphids differ in publication time and sex of the studied animals. Vintage studies come from 1836 to 1931, modern ones from 1970 to current. The sperm bundles can come from dissecting males or females. Sperm bundles from males do not show sperm cooperation, because they could disband before transfer. The evidence on sperm bundles from females is vintage and modern in equal measure. But it is rare and heterogeneous (see Supplement C).

5. Discussion

Sexual selection produced a great variety in the morphology of insect sperm, and sperm competition was a major cause of it (Pitnick, Hosken, and Birkhead 2009). Sperm competition can occur within an aphid clone because its sperm has two different kinships (Section 2). Furthermore, cystic spermatogenesis results in bundles of sperm cells within testes (see Supplement A). These are clonal for the X chromosome in aphids. This should facilitate the evolution of sperm cooperation in aphids. Sundry ecological factors can mix different clones and hence their males. This should increase sperm competition beyond that possible within clones (see Supplement B).

All this suggests that sperm competition should be as frequent in aphids as in related taxa (such as scale insects, Hemiptera: Sternorrhyncha). Yet the evidence for this phenomenon is rare and heterogeneous (see Supplement C). This dearth of compelling evidence is not a matter of course but requires an explanation.

5.1 Possible explanations for the inconclusive situation in aphids

First, the *Aphidoidea* may not form a uniform taxon in respect to sperm competition. Taxa without adaptations for sperm competition may experience none. Given the split sperm-kinship of a clone, this should only be true when mating is a rare event for average females. If they rarely encounter more than one male before oviposition, sperm competition will hardly occur.

Second, sperm conjugates found in female aphids may not compete with sperm from other males. Sperm transfer may be individual, to then form secondary conjugates within the female. Even human sperm cells cooperate under conditions of viscosity like the female reproductive tract's (Xiao et al. 2023).

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Supplement to:

'An unrecognized genetic predisposition for sperm competition in aphids'

A. Physiological preadaptation for sperm cooperation

In addition to the genetic predisposition for sperm competition to occur in aphids (see main text), insect spermatogenesis provides a physiological preadaptation for sperm cooperation. Cystic spermatogenesis results in the formation of sperm bundles within the male genital tract before they usually disband. Yet, in some species they do not disband, and sperm is transferred in coordinated bundles.

A stem-cell divides into a daughter stem-cell and a primary germinal cell. The latter becomes encysted within a sac formed by a single layer of epithelial cells. The germinal cell develops by synchronous division. At first, mitotic divisions multiply the number of germinal cells per cyst. At some point, varying with species, the germinal cells enter meiosis. The resulting cyst contains 2^n sperm cells, where n is the number of mitotic divisions plus the two meiotic divisions. The cysts elongate with the germ cells, which align in parallel (Virkki 1969; Phillips 1970; Dumser 1980; Heming 2018, chapter 1.3). The cyst develops into a cap surrounding the parallel sperm heads. Depending on taxa, the flagella are then free, surrounded by extracellular material or by a thin cytoplasmic sheath (Virkki 1969; Phillips 1970).

In aphids, one half of the meiotically produced final sperm cells do not receive an X chromosome and degenerate. Therefore, each aphid sperm-bundle only contains $2^n/2$ sperm cells. Yet these sperm bundles are clonal for their X chromosome. This situation differs from the one in which each spermatid in a bundle survives and matures into a functional sperm cell.

As only one sperm can fertilize an egg, the sperm bundles need to disintegrate, allowing the spermatozoa to disband, at some time before fertilisation. But the timing of this disintegration varies between taxa. If the sheath dissolves but the cap remains, the bundled sperm will be able to move together. Sperm conjugation can evolve by delaying the disintegration of sperm bundles beyond ejaculation. ‘The spermatozoa are inclined to remain in bundles, and in this state are expelled during copulation. These bundles are either root-like, bushy, string-like, sinuous, or worm-like’ (Packard 1898, 499). ‘Thus the progeny of one initial definitive gonium remains together until the bundle persists, e.g. at least until the sperm leaves the testis, but often until the genital organs of the female’ (Virkki 1969, 14). Delaying the disbanding of conjugates seems to be the simplest adaptation to sperm competition between ejaculates. In some cases, spermatozoa separate in the testes, but secondary conjugates form in the epididymis, seminal vesicles, or female reproductive tract (Pitnick et al. 2020, section IV.2).

Sperm conjugation is widespread (e.g., Virkki 1969; Miller & Kosztarab 1979; Moore et al. 2002; Pitnick et al. 2009; Higginson & Pitnick 2011; Pearcy et al. 2014; Nixon et al. 2016). This is even true for some Odonata (Siva-Jothy 1997; Åbro 1998), who are otherwise notable for removing sperm of competitors from the female’s genital tract with penis appendages. The morphological diversity of traits of sperm cooperation led to sundry technical terms, like spermatodesma (sperm-headband) or spermatozeugma (sperm-yoke). For simplicity, ‘sperm-conjugate’ will replace these technical terms.

Increased velocity could be the functionally-competitive advantage from conjugation (H. Moore et al. 2002; Pizzari and Foster 2008; Fisher et al. 2014), but the delay between pairings can thwart any chance for later sperm from subsequent ejaculates to overtake. However, many insects store transferred sperm in a special organ, the *spermatheca*. The competition between ejaculates should not occur in transit but at the storage site (Parker 1970). Conjugates from previous males might be better able to block the entrance to the receptacle than individual spermatozoa, and from later males might be better suited to force their way into it. There are other advantages from conjugation in competition with individual spermatozoa as well. For example, conjugates are known to be better at moving in viscous media, as typical in the female reproductive tract (H. D. M. Moore and Taggart 1995; Hayashi 1998; Pearcy et al. 2014). No matter whether, metaphorically speaking, the competition is racing, sumo, or parkours, conjugates seem to fare better.

B. Ecological factors facilitating sperm competition

Local mate competition (inbreeding) should bias sex allocation towards females, and aphid sex ratios are indeed mostly female biased (Foster 2002). Hence, the sex ratios of aphids suggest inbreeding. Inbreeding suggests that multiple pairings, if they do occur, will usually be with males of the same clone. Since the split kinship of sperm from the same clone has hitherto been ignored, the latter seemed to suggest that aphids are not the right field for sperm competition to play out. However, this assumption is not warranted. The differential kinship of sperm from the same clone opens the door for sperm competition to enter some aphid species (see above). Ecological factors leading to multiple mating should facilitate this. When multiple mating occurs, ecological factors mixing males form different clones should increase sperm competition even further.

Female polygamy has been frequently observed in aphids (e.g., Kozlowski 1991; Doherty & Hales 2002). Hence, multiple mating can be assumed to occur when conditions are favourable towards it. While some conditions may prevent multiple mating (e.g., rare males, old females with high egg pressure), several features of life history can increase the chance that males from different clones compete for the same females.

B.1 Terminology for aphid morphs

Female hatching from eggs are founding mothers or *fundatrices* (F). They are parthenogenetic bear larvae. These and a varying number of following generations reproduce in the same way. They are *viviparous* females (V). The last generation of parthenogenetic females produces the sexual morphs. They are *sexuparous* females (S). In a special life cycle, the sexuparae produce males and a special morph of parthenogenetic females. The latter will produce sexual females, they are *gynoparous* females (G). Finally, the sexual females are the only morph that lays eggs. They are *oviparous* females (O).

B.2 Clone mixing

The following factors will affect the mixing of aphid clones and hence the likelihood that males of different clones will compete.

I: Sexual females usually lay their recombinant eggs in clutches. If several eggs of a clutch survive and the hatching females stay together, the resulting colonies will consist of individuals from different clones.

II: The parthenogenetic offspring in spring and summer can be winged or wingless. Their flight will mix clones beyond the mixing that occurs through walking.

III: In species without host alternation, males are often winged and fly before mating. This will increase the likelihood that males from different clones come into competition.

IV: In some species host alternation occurs. Winged forms migrate between different summer and winter hosts. This can increase clone mixing or reduce it (see below).

V: In a special form of host alternation winged males and *gynoparous* females migrate to the winter host. That is, the two sexes migrate separately: males are winged and migrate, while sexual females migrate inside their gynoparous mothers (fig. 2).

B.2 Effects on sperm competition

Intuition suggests that the chance for competition among males from different clones should be low in species, where the parthenogenetic phase is extremely short, and clones remain small. For example, rosy leaf curling aphids (*Dysaphis devecta* (Walker)) and small willow aphids (*Aphis farinosa* Gmelin) produce three parthenogenetic generations. Sexual morphs occur in early summer (Hille Ris Lambers 1945; Forrest 1970; Emden 2013, 96).

The intuition that this precludes competition between males from different clones may still be wrong (see point I in 3.1, above). The sexual females lay eggs. As the ovipara of most species lay their eggs in clutches, their offspring will hatch in clutches. These *founding mothers* are recombinant but reproduces parthenogenetically. Only the following generations are clonal. A colony of *D. devecta* or *A. farinosa* will consist of the clonal offspring of founding mothers. Whenever a colony stems from more than one founding mother, it will consist of siblings and first cousins. Sometimes even of second cousins, when sibling sexual females produced a common clutch. Hence, short life cycles or small clones do not preclude competition between males of different clones in principle.

Factors II and III also increase clone mixing, but the different forms of host alternation (IV and V) affect sperm competition between clones differently. In IV, sexual morphs migrate inside their mothers (fig. 2, upper panel). Competition between clones can only occur, if these migrating mothers of the sexual morphs aggregate or their sons disperse before mating. In case V the sexes migrate separately (fig. 2, lower panel). Winged males fly on their own. Sexual females migrate inside their mothers (*gynoparae*). Here, males from different clones should be likely to compete. But males should also be rarer than females. The outbreeding selects for an even investment of the clone in its male and female functions. If the gynoparae are the clone's female function, this will produce an even ratio of gynoparae to males (Foster 2002, 255). Yet, if each gynopara produces several sexual females, female biased sex ratios will result despite outbreeding.

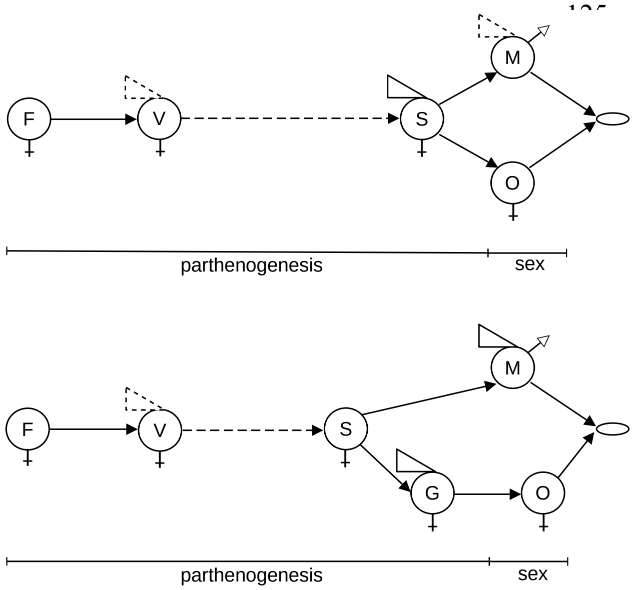

Figure 1: Host alternating aphids. Triangles indicate wings. Dashed wings indicate a polymorphism of winged and wingless morphs. Dashed arrows indicate variability in the number of generations produced. Dimension lines indicate the parthenogenetic and sexual phase of the life cycle. *Fundatrices* (F) hatch from eggs on the winter host and bear larvae through parthenogenesis. These and a varying number of following generations reproduce in the same way, *viviparous* females (V). Some V migrate to the summer host. *Sexuparous* females (S) produces sexual morphs at the season's end. In the simpler case [upper panel], S migrate to the winter host and produce *males* (M) and sexual females. The latter lay recombinant eggs (ellipse), they are *oviparous* females (O). In the complex case [lower panel], S produce M and *gynoparous* females (G) migrating to the winter host. G produce sexual females (O).

Part C: Evidence on sperm bundles in aphids

C.1 Vintage evidence (1836 – 1931)

Knowledge about sperm morphology in aphids is fragmentary and sometimes contradictory. This may be due to practical difficulties in obtaining sexual aphids, during the short period when they occur. Yet, the delay between consecutive copulas can be considerable (Kozlowski 1991, fig. 3), and the tip of the *aedeagus* (insect penis) seems to reach the spermathecal duct (Balbiani, 1870, art. no. 2, p. 20; Hales, 2005, p. 643).

Both observations suggest that sperm competition between different males, if it does occur, will take place at the spermatheca. Parker (1970, 527) already suggested as much for insects with sperm storage and multiple mating. Regrettably, publications that contain information about aphid sperm are rare. Birkhead & Montgomerie (2009) review three centuries of sperm research, but not that on aphids. However, a few studies have found evidence of aphid sperm conjugates (below).

C.1.1 Sperm conjugates from males. The following sources confirm sperm-conjugates in the testes of aphids, but do not tell whether sperm is also transferred as conjugates. Charles Morren (1836) dissected sexual aphids and described sperm from the testes of *Aphis persicae*, now *Myzus persicae* (Sulzer), as ‘rounded masses of very dense sperm’ and called them ‘globular spermatic animalcules’ (translation of Morren, 1836, p. 87). Carl von Siebold (1839) described *Aphis lonicerae*, now *Rhopalomyzus lonicerae* (Siebold), a honeysuckle-grass aphid that alternates between hosts: gynoparous females transport the sexual females from secondary hosts (grasses) to their primary (honeysuckle). The males also migrate and mate with the sexual daughters of the parthenogenetic gynoparae. Consequently, Siebold found winged males and viviparous females alongside wingless oviparous females (Siebold, 1839, column 307). He described sperm suspended in water as ‘oval tufts of hair’, which moved apart at one end to ‘change into a flower-bunch-shaped tuft of hair.’ He believed that Morren (1836) had seen the same, but had misidentified them as *animalcules spermatiques globulaires* (translation of Siebold 1839, column 308).

W. B. von Baehr (1908; 1909) observed *Aphis saliceti*, now *Aphis farinosa* Gmelin. He described sexuparae giving birth to males and oviparae (Baehr, 1909, p. 296), and a shortened life-cycle (Baehr 1909, 302f; see also Stevens 1906, p. 6; Kyber 1813, 15; Gillette and Bragg 1918, 91; Marcovitch 1924, 520). Beahr also dissected males and discovered sperm cysts (translation of Baehr 1909, 287).

Forrester W. Miller (1931) dissected males of an aphid found on cocklebur, describing their sperm as, ‘usually found clustered into bundles. Occasionally one is found isolated but this seems to be the exception rather than the rule’ (F. W. Miller 1931, 78). Unfortunately, he gave no description that would allow identifying the species of this cocklebur aphid, but he did mention the wings in males, yet their absence in sexual females (F. W. Miller 1931, 76).

C.1.2 Sperm conjugates from females. Édouard-Gérard Balbiani’s account of the sexual biology of aphids, both female and male, is particularly detailed (Balbiani 1869; 1870). Concerning the maturation of sperm within the males of various species (mostly *Siphonophora jaceae* Linnaeus, now *Uroleucon jaceae* Mordv.), his observations suggest that the sperm bundles disintegrate within a seminal vesicle of the vas deferens, ‘There, the filaments separate completely from each other, and curl up and often wind up like hairs’ (translated from Balbiani, 1869, p. 79f). Later, however, he also observed sperm within the spermathecae of mated females, ‘They appear, sometimes in the form of isolated filaments, entangled in each other, sometimes in the form of small bundles variously wound on themselves’ (Balbiani, 1870, art. no. 2, p. 19).

Thomas Henry Huxley studied aphids found on an oak tree in the London Zoological Gardens. His description (Huxley 1858, 203) fits that of a *Myzocallis* species. Upon dissecting females, he found ‘a multitude of large filiform spermatozoa bent upon themselves’ in the spermatheca (Huxley 1858, 205). The phrase ‘bent upon themselves’ could mean that the flagella of individual spermatozoa formed loops, or that sperm cells were conjugated.

Buckton’s take (in English) on Balbiani’s findings (in French) transformed Balbiani’s ambiguous evidence into a more definite interpretation that aphid spermatozoa move individually, ‘When the sperm-cells are mature the cysts break up or burst, and the filamented cells disentangle, and are then free to move down the vessels into the suitable vesicles’ (Buckton 1883, 4:128). Another reason why Balbiani’s observations were forgotten may have been due to his fallacious theorizing, thereby diminishing the credit in his descriptions. For example, he misinterpreted the tissue that nourishes the parthenogenetic eggs as male cells, and so mistook viviparous females as cryptic hermaphrodites that self-fertilize (Claparède 1867).

C.1.3 Life history traits and sperm competition. The above-mentioned species have several life history traits that increase the likelihood for sperm competition between clones. Males and gynoparous females of *M. persicae* migrate separately to winter hosts. The same is true for *R. lonicerae*. Though *Uroleucon jaceae* and *Myzocallis* species do not alternate hosts, their males have wings, as does Miller’s (1931) unidentified cocklebur aphid. In contrast, *A. farinosa* produces sexual morphs so early, that the clones remain small, but the males are wingless.

C.2 Modern evidence (1970 – current)

Since 1931, the knowledge about sperm morphology in other insects has advanced, but lagged in aphidology. Mazzini (1970) summarized his analysis of the structure of individual spermatozoa stating, ‘This sperm bears the conventional structure’ and Dallai, Gottardo, and Beutel (2016, 11) cited him stating that the ‘Sperm of Aphidoidea are of the conventional type’. However, this only covers the acrosome structure, the pattern of the tubules in the filament (9+9+2), and other features of individual spermatozoa of one species. It does not preclude the possibility that some sperm in some taxa are transferred in bundles.

Dagg (2002, 12) observed lengthy conjugates in the spermathecal duct and spherical ones in the spermatheca of female *Uroleucon cirsii* (Linnaeus) (see also Dagg 2003, 202). Hales (2005) rejected this for *M. persicae*, observing that the sperm cells within testes of adult males 'were not joined at the head, though they were during the last nymphal stage' (Hales 2005, 645), and concluding that the 'round objects in the spermatheca are likely to be an optical artefact' (Hales 2005, 643).

C.2.1 Sperm conjugates from males

Wieczorek and Swiątek (2008; 2009) observed 'bunches' of spermatozoa in the vas deferens of the males of *Phyllaphis fagi* Linnaeus, *Glyphina betulae* (Linnaeus), and *Anoecia (Anoecia) corni* (Fabricius). Vitale et al. (2009; 2011) recorded the development of sperm bundles in the testes of *Tuberculatus (Tuberculoides) eggleri* Börner and *Euceraphis betulae* (Koch). Yet, Vitale et al. (2011) reported that each bundle 'gradually becomes disorganized along the spermiduct, and the spermatozoa become free' (Vitale, Brundo, and Viscuso 2011, 277).

C.2.2 Sperm conjugates from females

Vitale and Viscuso (2015, 88) reported material inside the spermatheca of *E. betulae* that seemed to be a degenerating cap of a sperm bundle. Huang and Caillaud (2012, fig. 1C) found spherical sperm bundles inside the spermathecae of *Acyrthosiphon pisum* (Harris), where, after successful sperm transfer, 'the spermatheca was well inflated and included a small bundle of opaque white material.' Staining confirmed that it was a bundle of spermatozoa (Huang and Caillaud 2012, 5).

Wieczorek et al. (2019) reported 'chaotically oriented bunches of spermatozoa' in the testes and 'a dense substance in which spermatozoa are embedded' in the spermathecae of *A. pisum*. Finally, the testes of the arctic species *Acyrthosiphon svalbardicum* Heikinheimo were seen to contain 'bunches of ripe spermatozoa,' while the spermathecae contained 'dense aggregations of spermatozoa and male accessory gland secretions' (Wieczorek et al.

2020). Both observations seem to suggest a role for secretions from the spermatheca, or the male accessory glands, in secondary aggregations of sperm.

C.2.3 Life history traits and sperm competition

Monoecious species. *Uroleucon cirsii* does not alternate hosts, but the males have wings (Dagg and Vidal 2004, 232). The same is true for *Euceraphis betulae* and *Phyllaphis fagi*. Males of *Acyrthosiphon pisum* can be either winged or wingless. Huang and Caillaud (2012) had wingless males, whereas Wieczorek et al. (2019) had winged males. It has been mentioned that wings occur in *Tuberculatus (Tuberculoides) eggleri* (Börner) (Richards 1969, fig. 5).

Dioecious species. The sexes of *M. persicae* migrate separately to the winter host (both gynoparae and males are winged). The sexual morphs of *Anoecia (Anoecia) corni* migrate together, insider their sexuparous mothers: the males are wingless (Wieczorek and Świątek 2009, 154; Wieczorek, Płachno, and Świątek 2012, 306).

Shortened life cycles. *Glyphina betulae* does not alternate hosts, and its males are wingless, but sexual morphs already appear at the beginning of summer (Wieczorek and Świątek 2009, 154). *A. svalbardicum* has an extremely shortened life cycle, during which the founding mothers directly produce sexual morphs. Founding mothers can also produce some viviparous daughters under favourable conditions, but this has only rarely been observed (Wieczorek et al. 2020). Sperm competition between clones can still result, if more than one winter egg of a batch survives, and the recombinant founding mothers produce their offspring within the same patch.

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