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REVIEW

Reproductive polyphenism and its advantages in aphids: Switching between sexual and asexual reproduction



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Abstract

Reproductive polyphenism, which allows one genotype to produce sexual and asexual morphs, is an extreme case of phenotypic plasticity and is commonly observed in aphids. Aphids are typical species that switch these reproductive modes, and the pathway orientation is triggered by the environmental conditions (mainly photoperiod and temperature). The typical annual life of aphids includes a succession of parthenogenetic generations during the spring and summer and a single sexual generation in autumn. In this review, we describe how the environmental cues orientate the reproductive mode of aphids from photoperiodic perception to endocrine regulation, and how juvenile hormones may act on the target cells (oocytes) to initiate the gametogenesis and embryogenesis in sexual and asexual reproduction. We also discuss the paradox of sex, especially the advantages of sexual reproduction in aphids. With the recent development of genomic resources in aphids, many potential genes involved in the reproductive polyphenism will enter the public's awareness. In particular, we describe a novel RNAi method in aphids, which may provide a molecular technique for determining the developmental fate and multiple reproductive strategies.

Keywords: aphids, parthenogenesis, embryogenesis, gametogenesis, photoperiodism

1. Introduction

Polyphenism commonly observed in arthropods, is an interesting phenomenon, which allows a single genotype to produce several discrete phenotypes depending on the

environmental conditions such as temperature, photoperiod, diet, population density and the presence of predators (Gilbert 2001; Beldade *et al.* 2011). Such phenotype plasticity ultimately roots in the genomes and allows an individual organism to develop various morphological, physiological, or behavioral traits, which is considered as an evolutionary strategy (Uller 2008; Moczek 2015; Xue and Leibler 2018). Some insects usually switch their reproductive mode between sexual and asexual reproduction to manage the seasonal changes in temperature, day length and host availability (Matsuka and Mittler 1979; Margaritopoulos and Tsitsipis 2002; Poupoulidou *et al.* 2006; Razmjou *et al.* 2010; Peng *et al.* 2017). The sexual reproduction dominated in eukaryotic life is initiated from the fusion of male and female

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gametes produced through meiosis, whereas the asexual reproduction is achieved without meiosis and fertilization. These two reproductive models in one species have been observed in aphids (Vorburger *et al.* 2003; Ollivier *et al.* 2012), termites (Matsuura *et al.* 2009; Yashiro and Matsuura 2014), ants (Pearcy *et al.* 2004; Fournier *et al.* 2005), honey bees (Gerber and Klostermeyer 1970; Beekman *et al.* 2011), beetles (Lachowska *et al.* 2008; Perotti *et al.* 2016), locusts (Hamilton 1953; Little *et al.* 2017) and moths (Stunnikov 1975; Xu *et al.* 2004).

Aphids belonged to the Order Hemiptera (sucking bugs) are major agricultural pests causing great economic loss by ingesting plant sap for nutrition, and transmitting viral diseases in many crops (Le Trionnaire *et al.* 2008). The rising global atmospheric CO₂ level may promote the aphid outbreak (Chen *et al.* 2004; Guo *et al.* 2013; Sun *et al.* 2013), which is likely related with the high reproductive capacity of aphids. The aphids share the ability of alternative pathways of sexual and asexual reproduction, and nearly 5000 aphid species have been reported to share apomictic parthenogenesis (clonal or asexual reproduction) as the main or exclusive mode of reproduction (Ogawa and Miura 2014). As shown in Fig. 1, their typical annual life cycle is characterized by a succession of several parthenogenetic generations (viviparous parthenogenetic females) during the spring and summer and a single sexual generation (males and oviparous females that mate and lay the diapausing eggs to resist winter) in autumn (Simon *et al.* 2002; Tagu *et al.* 2005; Ogawa and Miura 2014). Cyclical parthenogenesis is acquired by a common sexual ancestor (about 250 million years ago) and predominates in aphids, whereas only a small portion of aphids lose the sexual phase and become strict obligate parthenogens (Moran 1992; Hales *et al.* 1997). There are two other interesting phenomena in aphid reproduction: (1) some obligate parthenogens retain the capacity for male production, which is common to many sexual organisms and (2) the rest have obviously evolved a strategy that is intermediate lineages between sexual and asexual lineages, and the variable investment of sex can be observed in the same species or even in the same population (Rispe and Pierre 1998; Simon *et al.* 2002). Thus the reproductive mechanism of aphid is mysterious, which makes them a good model to study the ecology and evolution of sex.

In the present review, to illustrate how aphids measure the night length in the cephalic region and transduce the signal to the target cells in ovaries to alternate their reproductive modes, we discuss: (1) the environmental cues for switching reproductive modes; (2) the perception of photoperiodic changes; (3) the endocrine regulation; (4) the gametogenesis and embryogenesis in sexual and asexual morphs; and (5) the paradox of sex (the advantages

of sexual reproduction), and summarize the hypothetic photoperiodic determination of reproductive mode in Fig. 2. In particular, we introduce a novel RNAi method to down-regulate the gene expression in aphids, which may greatly promote the identification of pathway genes for switching reproductive modes.

2. Environmental cues for switching reproductive modes

In most studied aphid species, long-night seems to be a major environmental cue for switching reproductive modes. The regulation of sexual reproduction by night length was first confirmed by Marcovitch (1923, 1924) in aphids, which is the first report of photoperiodic induction in animals. He showed that the exposure of *Aphis forbesi* to short-day length promoted the production of sexual morphs. Since then, the regulation of reproductive modes by photoperiodic changes has been demonstrated in *Phorodon humuli* (Campbell and Tregidga 2005), *Acyrtosiphon pisum* (Lamb and Pointing 1972), *Myzus persicae* (Matsuka and Mittler 1979; Margaritopoulos and Tsitsipis 2002) and *Megoura viciae* (Hardie 1990). The sexual reproduction is induced by scotophases longer than 9–10 h, and this threshold corresponds to early autumnal conditions. Aphid photoperiodic responses clearly involve a clock that is used to measure the scotophase length rather than the photophase length, and a counter mechanism that integrates the information from the clock may exist to induce the polyphenism (Lees 1973; Saunders 1981). The most well-studied circadian clock genes show different expression levels between scotophase and photophase (Yan *et al.* 2013, 2017, 2019), but the role of circadian clock genes in response to the seasonal photoperiodism is still unclear. With the help of genomic resources for aphids, this question may be answered in future.

Although the photoperiodic changes direct the reproductive mode in aphid, the temperature can modulate this photoperiodic response. In some aphid species, the high temperature can override the long night. For example, the critical photoperiod for sexual production of *A. pisum* is shorter at the higher temperature (from 13 h at 15°C to 12.5 h at 20°C) (Lamb and Pointing 1972). The production of sexual morphs is higher at 12°C than at 17°C in most *M. persicae* clonal lineages (Poupoulidou *et al.* 2006). The activity of male production is higher at 17°C than at 20°C in the Utsunomiya clone of *A. glycines* under 10 h L:14 h D (Oka *et al.* 2018). In many aphids with coexisting sexual and asexual reproduction, the obligate parthenogenesis is more common at lower latitudes, confirmed in *Sitobion avenae* (Dedryver *et al.* 2001), *Rhopalosiphum padi* (Delmotte *et al.* 2002), *A. pisum* (Kanbe and Akimoto 2009) and *M. persicae*

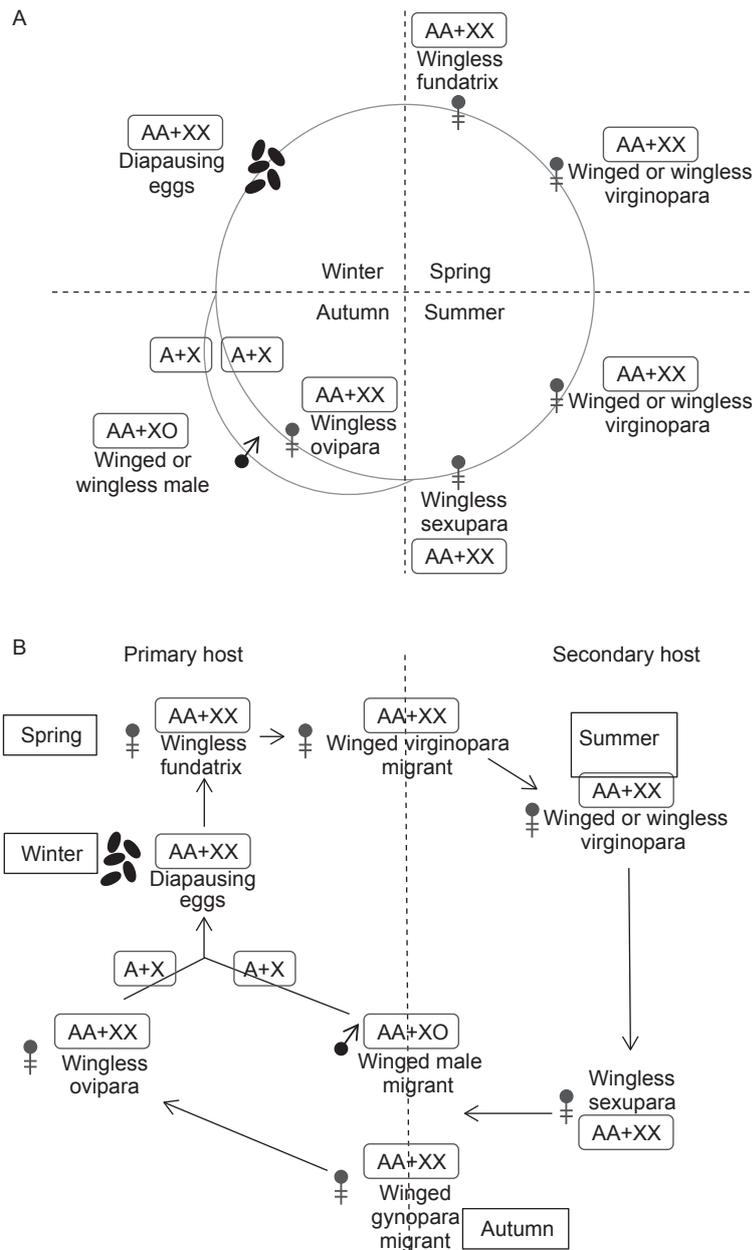


Fig. 1 Typical annual life-cycle of aphids. Aphids employ the XO sex-determination system. The male randomly loses one X chromosome during the maturation division and possesses only one X chromosome. A, schematic diagram of a typical life-cycle of nonhost-alternating aphid. In spring, the wingless fundatrix is hatched from the diapausing egg to produce the winged or wingless virginopara. By late summer, the change in photoperiod and temperature triggers the production of wingless sexupara that produces sexual morphs. Winged or wingless male and wingless ovipara mate in autumn and ovipara lays overwintering eggs. B, schematic diagram of a typical life cycle of a host-alternating aphid. In spring, the wingless fundatrix is hatched from the diapausing egg on the primary host, and then it produces first wingless, then winged virginopara that migrates to the secondary host. By late summer, the change in photoperiod and temperature triggers the production of wingless sexupara that produces sexual morphs. Winged male and winged gynopara migrate to the primary host. The gynopara produces the wingless ovipara that mates with winged male and lays the overwintering eggs.

(Blackman 1974), and this pattern appears to be primarily determined by the combined effects of photoperiod and temperature. However, the latest research showed that all tested obligate parthenogenetic lineages of *M. persicae*

lost the functional *hsp90* genes (severe mutations), and the *hsp90*-inhibitor made some cyclical parthenogenetic lineages lose their ability to produce sexual morphs, revealing the great importance of functional *hsp90* genes

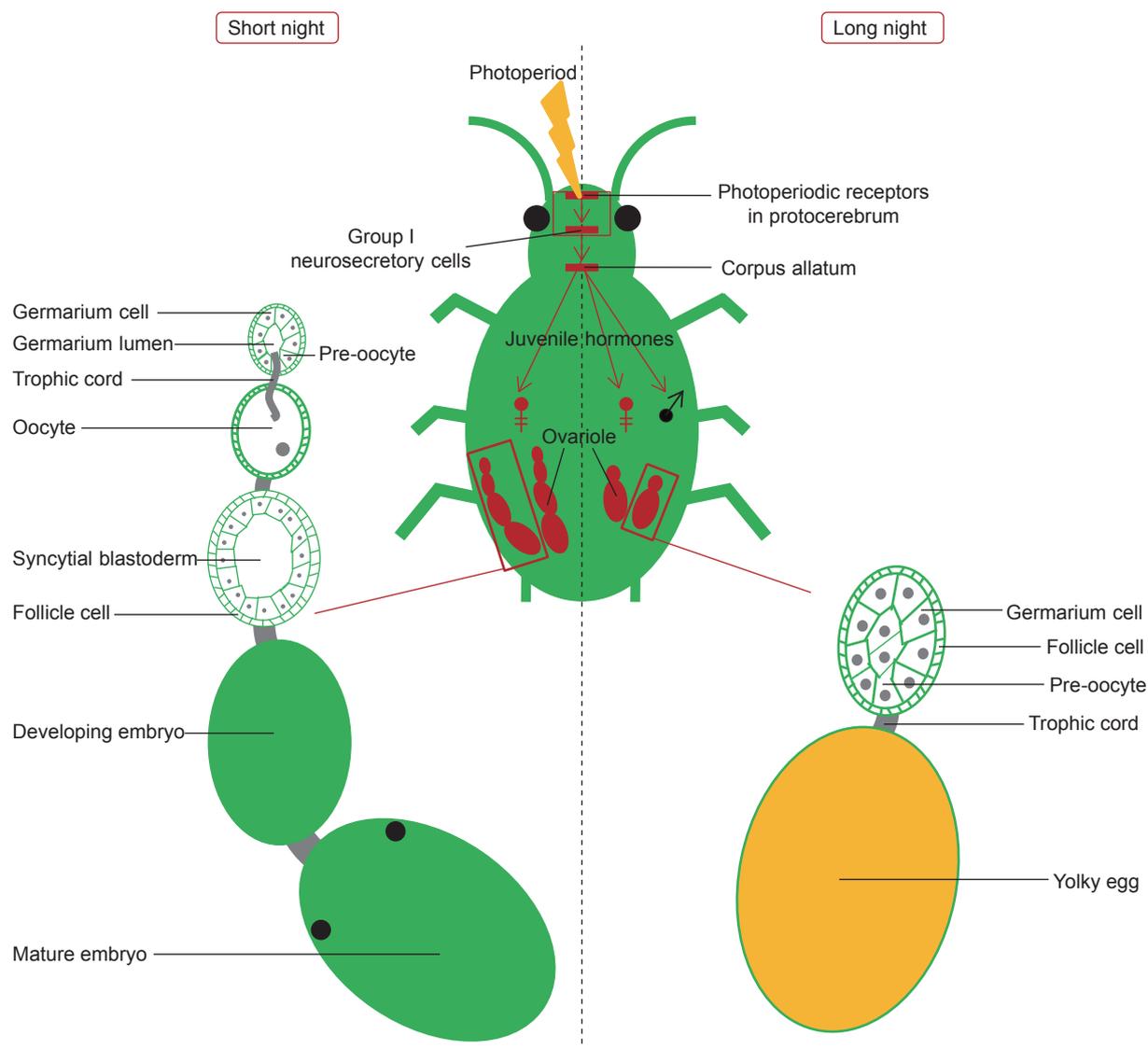


Fig. 2 Photoperiodic determination of reproductive mode in aphids. After the stimulation by photoperiod (short or long night), the photoperiodic receptors activate the Group I neurosecretory cells in protocerebrum, which in turn produce the neurohormones. The neurohormones act on the corpus allatum to lead a change in juvenile hormone (JH) titre, which switches the fate of oocytes in the germarium. Under short night (left side), the JH titre is high enough to trigger the asexual reproduction in aphids. Under long night (right side), the JH titre is lower, which results the sexual reproduction in aphids.

in cyclical parthenogenesis (Mandrioli *et al.* 2018).

In some aphid species, the host availability may also influence the sexual reproduction. The *A. pisum* lineages show a significantly higher investment in sexual reproduction on annual crops than perennial hosts (Frantz *et al.* 2006). The host-alternating aphids tend to be highly specialized on the primary host (usually perennial hosts), on which the copulation occurs and the frost-resistant eggs are laid. Thus, the cyclical parthenogenesis happens only when the special host is available, whereas the obligate parthenogenesis of the same species is free from this constraint (Sandrock *et al.* 2011). The sexual morphs of *Dysaphis devectora* are

produced at the cessation of host plant shoot growth (Forrest 1970), revealing that the physiological changes of the hosts also influence the sexual reproduction.

3. Perception of photoperiodic changes in cephalic region

Vision plays numerous key roles in the performance of insect behaviors, such as searching for food and potential mates, avoiding predators and unsafe environment, and other specific behaviors (Kelber *et al.* 2003; Langham 2004; Zhao *et al.* 2009; Yan *et al.* 2014a; Li *et al.* 2015).

The opsins located in compound eyes are responsible for detecting and converting light, and their expression is regulated by the circadian clock (Yan *et al.* 2014b; Liu *et al.* 2018). Nevertheless, the opsins in compound eyes are not the main center for photoreceptor, and the aphids are able to measure the night length in the cephalic region. The site of the photoperiodic receptors for switching reproductive modes in *Megoura viciae* has been previously identified by exposing the aphids to supplementary periods of localized illumination, showing that the photosensitivity is confined to the cephalic region, especially the dorsum of heads rather than the compound eyes or optic lobes (Lees 1964). All wavelength lights between 370 and 800 nm can be transmitted through the cuticle to brain (Hardie *et al.* 1981). The protocerebrum beneath the cuticle is highly photosensitive, and the photoreceptors for sensing the seasonal changes are thus extra optic.

Antibodies directly against the invertebrate and vertebrate opsins and phototransduction proteins were tested on *M. viciae*, but the organs other than brain (e.g., the compound eyes) were also labeled (Gao *et al.* 1999), implying that the cloned opsin genes may not be involved in the photoperiodic response, but rather in visual responses. Gao *et al.* (2000) further sequenced the aphid brain opsin, but the attempt was unsuccessful. In addition, the pea aphid cuticular protein genes are regulated by the night length, suggesting a remodeling of the head cuticle under the different photoperiods, which may accompany photoperiodic signal transduction (Le Trionnaire *et al.* 2007; Cortés *et al.* 2008). Identification of the photoreceptor center for sensing the seasonal changes in brain is a fascinating task. Lesions destroying the Group I neurosecretory cells (NSC) in the protocerebrum disables the production of asexual morphs under long-day conditions in *M. viciae*, but the extensive damage to the other NSC groups (the compound eyes and optic lobes) has no such effect (Steel and Lees 1977), revealing that a small area of the protocerebrum containing NSC is indispensable for the photoperiodic mechanism. The NSC is not the photoperiodic photoreceptor (Gao *et al.* 1999), and the photoreceptors for photoperiodic response and NSC involved in neurosecretion are two different sequential players of the photoperiodic signal transduction (Tagu *et al.* 2005). In non-mammalian vertebrates, the photoreceptors in the deeper regions of the brain mediate the seasonal changes in physiology and behavior, and the OPN5-positive CSF-contacting neurons in the paraventricular organ of the quail mediobasal hypothalamus have been reported to shown intrinsically photosensitive, involved in the regulation of seasonal reproduction (Nakane *et al.* 2014). Early transduction of the photoperiodic signal may occur through NSCs in protocerebrum, but it is not clear whether they are directly connected to the ovaries.

4. Endocrine regulation of reproductive mode

Juvenile hormones (JHs) involved in many physiological functions have been demonstrated to regulate various insect polyphenisms, such as the wing polyphenism, body-color polyphenism, male polyphenism and caste polyphenism (Zera and Denno 1997; Emlen and Nijhout 1999; Tanaka 2001; Miura 2005). Since the corpora cardiaca is connected to the brain and corpus allatum that produces and secretes JHs, it may constitute both release and transit sites for brain neurosecretory products (Tagu *et al.* 2005). Interestingly, the corpus allatum volume is regulated by the night length, but it is not directly correlated with JH titre (Hardie 1987a). JHs are candidate molecules for the transduction of the photoperiodic signal from the brain to ovarioles. Although there are several types of JHs exist in insects, only one type (JHIII) has been reported in aphids in a small amount (Hardie *et al.* 1985; Westerlund and Hoffmann 2004). The pea aphids reared under long-night conditions have lower JHIII titre than those under short-night conditions, and the expression level of *JH esterase 1* gene that is known to degrade JH is higher in aphids reared under long-night conditions (Ishikawa *et al.* 2012), suggesting that the up-regulation of the JH degradation pathway may result in the production of sexual morphs. The topical application of JHs or their analogues under long-night conditions leads to the production of parthenogenetic females instead of sexual morphs in *M. persicae* (Mittler *et al.* 1979), *A. fabae* and *A. pisum* (Mittler *et al.* 1976; Corbitt and Hardie 1985; Hardie and Lees 1985). For instance, starvation, decapitation and precocene III treatments inhibit the embryonic oocyte development, and JH treatment reverses this inhibition (Hardie 1987b). Besides JH, the melatonin usually involved in the transduction of day length in vertebrate, also influences the reproductive mode in aphids. The melatonin-fed *A. pisum* under 16 h L:8 h D produce males and virginoparous/oviparous intermediate females, which occurs only under short-day conditions or around critical night-length (Gao and Hardie 1997).

The signal transduction of photoperiodic changes to secretory organs to perform endocrine regulation is still poorly understood. The most likely mechanism may be that: (1) the photoperiodic changes are perceived by the protocerebrum to activate the neurosecretory cells; (2) the neurosecretory cells produce the neurohormones to act on corpus allatum to produce and secrete JHs; (3) JHs are transported along axons connected to the abdominal structures and, perhaps, even to the ovarioles. However many issues remain unsolved including the types of JHs regulating the reproductive mode, the JH receptors and

the putative chemical mediators that interact with JHs. With the progress of molecular techniques, such as the transcriptional profiling and nanocarrier-mediated RNA interference (see below), the important role of JHs in the reproductive polyphenism may be illustrated.

5. Gametogenesis and embryogenesis in sexual and asexual morphs

The environmental signal is perceived and transduced to the final target organ (ovary) to produce the sexual or asexual morphs. Similarly to most insects, each female aphid possesses two functional ovaries, and their general organization and embryonic development are similar between sexual and asexual aphids. Each ovary contains several telotrophic meristic ovarioles, each consisting of a germarium and a chain of follicular chambers. In each fully-formed germarium, there are 32 oogonial cells divided by the germ cell, half of them developing into nurse cells (or trophocytes) and half into oocytes (Büning 1985). The pre-oocytes are expelled from the germarium toward the first follicle chamber to become the oocytes, and the oogonial cells located in the posterior part of the germarium condense their chromosomes and become the pre-oocytes (Chang *et al.* 2006, 2007; Le Trionnaire *et al.* 2008; Bermingham and Wilkinson 2009). The pre-oocytes are released one by one into the follicle chambers in this way, and each follicle chamber contains the egg or embryo at different developmental stages.

In the case of sexual reproduction of aphids, the meiosis is to release one haploid oocyte and three degenerated polar bodies, which is similar with other insects (Tagu *et al.* 2005). The oocytes in follicle chambers enter a growth phase with accumulation of yolk in their cytoplasm, and then are fertilized when passing into the oviduct beside the spermathecae. The fertilized eggs filled with yolk are approximately 1 mm long, and they darken from the posterior towards the anterior pole due to the deposition and tanning of a cuticle by the serosa (Miura *et al.* 2003). When the embryo completes anatrepsis and is fully segmented, it enters diapause, a period of arrested development (Le Trionnaire *et al.* 2008). In the case of parthenogenetic aphids, the embryo develops within ovariole from a diploid oocyte after a single maturation division (the mitosis that produces one 2n polar body and one 2n oocyte without recombination) (Tagu *et al.* 2005). The first mitotic division of the oocyte occurs immediately after the parthenogenetic embryogenesis, and the number of embryo per asexual female is much higher than those in sexual females (Miura *et al.* 2003). The oocytes of asexual aphids lack the asters provided by the male gamete in sexual aphids but they can self-organize the microtubule-based asters (Riparbelli

et al. 2005).

Obviously, the oocyte fate toward sexual or asexual differentiation is decided very early, perhaps at the oocyte or pre-oocyte stage. The parthenogenetic embryos express a large number of tissue-specific genes and some highly expressed genes do not share any similarity with other sequences in GenBank (Sabater-Muñoz *et al.* 2006), indicating the differentiation process specific to parthenogenetic embryogenesis in aphids. Srinivasan *et al.* (2014) observed the similar expression patterns of all meiosis genes between asexual and sexual ovaries of *A. pisum*, with the only exception for *Spo11*. The asexual aphids accumulate unspliced transcripts of *Spo11*, whereas the sexual aphids accumulate primarily spliced transcripts. The *Spo11* protein creates double-strand DNA breaks that initiate the recombination in meiosis, and less *Spo11* protein in asexual aphids may prevent the inappropriate recombination, DNA damage and chromosome pairing, revealing that the modification of *Spo11* gene activity may play an important role in switching the reproductive modes.

6. The paradox of sex: advantages of sexual reproduction

The cyclically parthenogenesis theoretically has a two-fold reproductive disadvantage compared to the asexual reproduction, and the paradox of sex (the prevalence of cyclical parthenogenesis over obligate parthenogenesis) seems more obvious in aphids than most other organisms (Bulmer 1982; Rispe and Pierre 1998). The first advantage of sex maintenance may be that only cyclical parthenogenetic aphids produce the eggs that are resistant to the cold climate, whereas the sexual aphids do not produce eggs, and are hence susceptible to the cold climate. There are two exceptions that some *Fordinae* aphids produce sexual eggs that do not overwinter, and the parthenogenesis is oviparous in *Adelgidae* and *Phylloxeridae* aphids (Simon *et al.* 2002). The sexual lineages may predominate in cold climate, whereas they are likely to be replaced by asexual lineages in warm climate, and the asexual reproduction is deemed to be a superior reproductive output (Rispe *et al.* 1998; Dedryver *et al.* 2001). The sexual eggs are not only cold resistant, but may be also adapted to the drought, and the eggs can be easily dispersal by seeds.

The second advantage of sex maintenance may be related with the genome purging and high diversity. The reproductive mode of aphids determines their genetic diversity to some extent, which is confirmed by genetic markers such as mitochondrial DNA and microsatellite. For example in *R. padi*, the cyclically parthenogenetic lineages possess different mitochondrial DNA than most asexual clones (Simon *et al.* 1996), and the microsatellite

results indicate that the sexual lineages have high allelic polymorphism and heterozygote deficits, whereas the asexual lineages show less allelic polymorphism but high heterozygosity at most loci (Delmotte *et al.* 2002), which is similar to the results of *A. pisum* (Kanbe and Akimoto 2009). A great genetic diversity is usually found in cyclically parthenogenetic lineages (Sunnucks *et al.* 1997; Gómez and Carvalho 2000), whereas the obligate asexual lineages possess fewer genotypes (Fenton *et al.* 1998; Wilson *et al.* 1999; Haack *et al.* 2000). A significant acceleration of deleterious mutation accumulation is also observed in the asexual *Tuberolachnus salignus* (Normark and Moran 2000). By generating genotypic diversity, sexual aphids can reduce the intraspecific competition and help aphids to escape parasites. Nevertheless, the asexual aphids also possess mitotic recombination, which occurs at a low rate (Wilson *et al.* 1999). The males produced by asexual lineages can mate with females produced by sexual lineages to shelter the asexual genes in diapausing eggs for overwintering, and there are some evidences of gene flow between sexual and asexual lineages in nature (Rispe *et al.* 1999), which is benefit for the genetic diversity of asexual lineages.

7. Prospect of a novel RNAi method in aphids

Many issues are not clear in the switch of reproductive modes in aphids: (1) the photoreceptors in protocerebrum are unknown, (2) the regulation of photoperiodism by circadian clock is debated, (3) the role of JH in photoperiodic transduction is not clear, and (4) the genes and proteins involved in the switch of reproductive modes need to be further identified. The sexual and asexual aphids use the same set of genes, and the mechanism of reproductive polyphenism need more molecular proofs. With the establishment of the International Aphid Genomics Consortium and sequencing of aphid genome, many potential genes involved in the reproductive polyphenism will enter the public's awareness. RNA interference (RNAi) allows the transient knock-down of mRNA, which is a good method to analyze the gene function. To our knowledge, there are still some challenges in RNAi for Hemiptera such as the dsRNA delivery, dsRNA persistency and RNAi machinery (Allen and Walker 2012; Christiaens *et al.* 2014; Christiaens and Smagghe 2014), and thus this method has not yet been adapted to promote the effective and high-throughput mutagenesis in aphids.

The toughest problem to apply RNAi in aphids is the dsRNA delivery method. Considering the limitations of existing dsRNA delivery systems (e.g., the mechanical injury for microinjection method and the low penetration capacity of dsRNA into the body wall for soaking method), the oral

feeding of dsRNA seems more practical. We previously introduced a nanocarrier to establish a novel dsRNA delivery method through oral feeding. The nanocarrier is a cationic dendrimer that consists of peripheral amino acid functionalized arms. The dendrimer can condense random nucleus acid with length ranging from 20–2 000 bp into nanocarrier/nucleus acid complexes that are easy to be uptaken through endocytosis (Win and Feng 2005; Yin *et al.* 2008; Xu *et al.* 2014). Our nanocarriers deliver the dsRNA to down-regulate the target gene expression through oral feeding, resulting in an obvious phenotype with chewing mouthpart pests (He *et al.* 2013; Liu *et al.* 2014; Shen *et al.* 2014; Xu *et al.* 2014). However, the feeding method does not seem suitable for sap-sucking aphids that usually have a small oral dose of dsRNA (Yu *et al.* 2013; Christiaens and Smagghe 2014). Recently, we have established a transdermal delivery system in *A. glycines* with the help of the nanocarrier and detergent (Fig. 3), which helps the dsRNA to penetrate the body wall into the hemocoel and

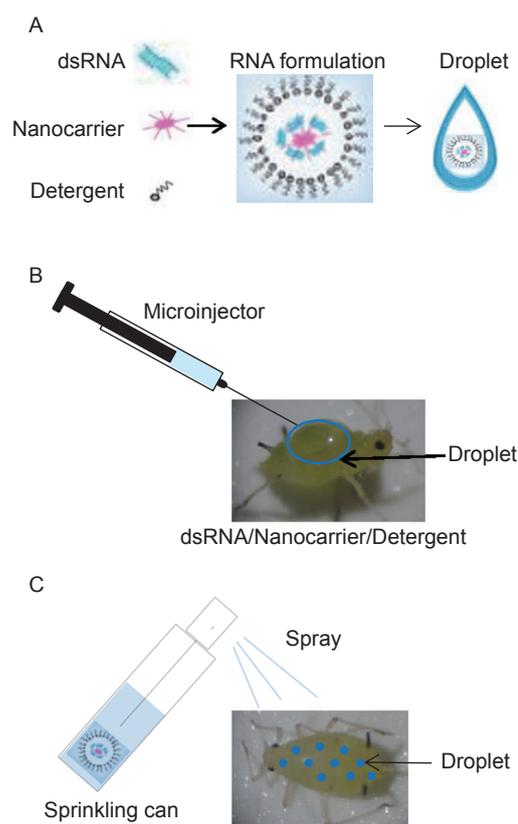


Fig. 3 Schematic diagram of nanocarrier mediated RNA interference in aphids. A, the schematic diagram of transdermal dsRNA delivery system. The dsRNA/nanocarrier/detergent formulation is performed by two mixture operation steps, and the amphiphilic droplets can spread on the aphid notum with the help of detergent. B, the schematic diagram to conduct RNAi through the topical application. C, the schematic diagram to conduct RNAi by spraying method.

then spread into various tissues (Zheng et al. 2019; Yan et al. 2020). The amphiphilic periphery detergent reduces the surface tension of hydrophilic nanocomplex droplets and helps dsRNA/nanocarrier complexes fast adhere to the notum of aphids. Our novel dsRNA delivery system achieves a high RNAi efficiency (95.4%), just by conveniently dropping or spraying dsRNA/nanocarrier/detergent formulation on the notum of aphids to avoid the postoperative side effects (Zheng et al. 2019), which will provide an excellent method to identify functional genes and greatly promote the identification of pathway genes involved in the switching of reproductive modes in aphids.

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