

Postprint: Differentiation Patterns of Sexual Morphs in the Green Pea Aphid under Different Photoperiod Conditions

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Date: 2017-11-08T00:00:00+00:00

Abstract

To elucidate the population reproductive strategy and sexual morph differentiation patterns of pea aphids in response to photoperiod changes, this study investigated sexual morph differentiation in the offspring (G3) of the green morph of pea aphid after two consecutive generations of photoperiod induction (G1 and G2) under four photoperiod conditions (8L:16D, 10L:14D, 12L:12D, and 14L:10D). The results showed that in the Lanzhou region, sexual females of pea aphids were all wingless, while males were all winged. Photoperiod had a significant effect on sexual morph differentiation in pea aphids ($P < 0.05$). Under the 14L:10D photoperiod, no sexual morphs differentiated in the pea aphid offspring; under the three photoperiods of 12L:12D, 10L:14D, and 8L:16D, sexual morphs differentiated, and as photoperiod decreased, the percentage of males in G3 gradually declined while the percentage of sexual females gradually increased. Under the 12L:12D photoperiod, the proportion of males was highest (30.39%), while the proportion of sexual females was lowest (55.67%); under the 10L:14D photoperiod, the proportion of viviparous aphids in G3 was highest while the proportion of sexual morphs was lowest; under the 8L:16D photoperiod, no male differentiation occurred while the proportion of sexual females was highest (90.76%). Under the same short-day condition, the reproductive age of G1 had a significant effect on sexual morph differentiation in G3 ($P < 0.05$), and sexual morph differentiation exhibited a distinct temporal sequence, i.e., sexual females were produced first, followed by males, with viviparous aphids being produced concurrently during the transition from sexual females to males. This indicates that the critical photoperiod for sexual morph production in the green morph of pea aphid ranged between 12-14 h, with relatively longer photoperiods favoring male production and shorter photoperiods resulting in higher proportions of sexual females. This demonstrates that photoperiod variation is an important factor in reproductive mode transition and sexual morph differentiation in

pea aphids, and that the duration of photoperiod treatment experienced by the parental generation influences sexual morph differentiation in the offspring.

Full Text

Abstract

In order to clarify the population reproduction strategy and differentiation patterns of sexual morphs in the green form of pea aphid (*Acyrtosiphon pisum*) in response to photoperiod, we examined sexual morph production after two continuous generations of photoperiodic induction (G1 and G2) under four photoperiod regimes (8L:16D, 10L:14D, 12L:12D, and 14L:10D). The results showed that oviparae (sexual females) in the Lanzhou population were all wingless, while males were all winged. Photoperiod had a significant effect on sexual morph differentiation ($P < 0.05$). No sexual morphs were produced under the 14L:10D photoperiod, but substantial differentiation occurred under the other three short-day regimes. As day length decreased, the proportion of males in G3 gradually declined while the proportion of oviparae increased. The highest male percentage (30.39%) occurred under 12L:12D, which also yielded the lowest ovipara percentage (55.67%). Under 10L:14D, viviparae (asexual females) reached their highest proportion while sexual morphs were least abundant. Under 8L:16D, no males were produced and oviparae reached their maximum proportion (90.76%). Under the same short-day conditions, the reproductive timing of G1 significantly affected sexual morph differentiation in G3 ($P < 0.05$). Sexual morph differentiation followed a distinct temporal sequence: oviparae appeared first, followed by males, with viviparae being produced during the transition between sexual female and male production. These findings indicate that the critical photoperiod for sexual morph induction in the green pea aphid lies between 12 and 14 hours of light. Relatively longer day lengths favored male production, while shorter day lengths promoted higher proportions of oviparae. Photoperiod change is thus a critical factor regulating reproductive plasticity, and the duration of photoperiodic exposure in maternal generations influences sexual differentiation in offspring.

Keywords: *Acyrtosiphon pisum* (Harris); Green form; Photoperiod; Male aphid; Ovipara; Vivipara

Introduction

Phenotypic plasticity serves as a key adaptive mechanism for organisms responding to environmental heterogeneity, making it a central focus in studies linking ecology and evolution [1-2]. Aphids exhibit extensive non-genetic polyphenism, including variations in body color, wing morphology, and reproductive mode [3]. Among these, reproductive polyphenism represents a paradigm of aphid evolution and environmental adaptation [4]. Under long summer day lengths, aphids reproduce asexually through parthenogenetic viviparity, enabling rapid population growth that severely impacts agricultural production. As autumn

progresses and environmental conditions deteriorate—characterized by declining temperatures and shortening photoperiods—parthenogenetic females perceive these changes and produce sexual morphs (males and oviparae) in subsequent generations, allowing the population to overwinter as diapausing eggs. The production of sexual morphs thus not only facilitates survival through adverse conditions but also promotes gene exchange and maintains genetic diversity within populations, playing a crucial role in aphid survival and evolution [5-7]. Artificial induction of sexual morphs under controlled conditions provides an important tool for investigating aphid polyphenism and population genetics.

The pea aphid (*Acyrtosiphon pisum*), also known as the pea louse, is a major pest of leguminous crops and forages in temperate regions [8-9]. Its widespread polyphenism, cyclical parthenogenesis, and holocyclic life cycle on a single host make it an ideal model organism for developmental and evolutionary biology research [4,9]. In its annual life cycle, pea aphids produce one generation of sexual morphs (males and oviparae) when photoperiod shortens and temperature drops. Oviparae are typically wingless, while males exhibit wing dimorphism. Smith and MacKay [10] reported three male wing morph types in pea aphids: exclusively winged, exclusively wingless, and mixed populations, with geographic variation in wing morph differentiation. Caillaud et al. [11] demonstrated through Mendelian genetics that male wing polymorphism is controlled by alleles at the *api* locus on the X chromosome. Lees [12] and MacKay [13] investigated sexual morph differentiation patterns under various photoperiods in English and Canadian pea aphid populations, revealing significant geographic variation. Chinese researchers have studied sexual morph induction through low temperature and short-day photoperiods in the peach aphid (*Myzus persicae*), cotton aphid (*Aphis gossypii*), and soybean aphid (*Aphis glycines*) [14-17], but few reports exist on sexual morph differentiation in pea aphids. Field surveys indicate that while the red form of pea aphid is increasing in northwestern China, the green form remains the dominant population and appears earliest each spring [18-20]. Therefore, this study focused on the green form to investigate how photoperiod affects sexual morph differentiation, aiming to clarify the conditions and patterns of sexual morph production, understand population reproductive strategies in response to photoperiod changes, and provide a foundation for future population genetics research on pea aphids in northwestern China.

Materials and Methods

1.1 Test Insects

Green-form pea aphids were collected from alfalfa experimental plots at Gansu Agricultural University in Lanzhou and maintained on potted broad bean (*Vicia faba* 'Linca 2') plants at (22±1)°C, 16L:8D photoperiod, and 60% relative humidity for multiple generations before use.

1.2 Experimental Methods

Sexual morph induction experiments were conducted in artificial climate chambers at 15°C with four photoperiod treatments: 8L:16D, 10L:14D, 12L:12D, and 14L:10D. The experimental protocol followed Wang et al. [17] with minor modifications; aphids were reared individually on detached leaves in Petri dishes [21]. First-instar nymphs (G1) produced within a 2-hour window were collected from the stock plants (representing different clones when possible) and placed on broad bean leaf discs in Petri dishes, which were then transferred to the appropriate climate chambers for two continuous generations of induction. When G1 individuals matured and began reproducing, nymphs (G2) produced on days 2, 7, and 12 of the reproductive period were collected (10 nymphs per day) and reared singly in new Petri dishes. Once G2 began reproducing, their offspring (G3) were collected daily until all G2 individuals died. All G3 nymphs were reared to adulthood on fresh leaves for morph identification. Morphs were distinguished by dissecting and examining the reproductive system: G3 individuals containing red-eyed embryos (winged or wingless) were identified as parthenogenetic viviparae, those with yellow eggs and wingless were oviparae, and those with black claspers (winged or wingless) were males [11,22]. The proportions of each morph were then calculated.

1.3 Data Processing

All data were analyzed using Microsoft Excel 2003 and SPSS 16.0. Errors in tables are presented as mean \pm standard error (mean \pm SE). One-way ANOVA was performed using SPSS 16.0, with Duncan's multiple range test for post-hoc comparisons.

Results

2.1 Effects of Different Photoperiods on Sexual Morph Differentiation

As shown in Table 1, photoperiod had a significant effect on sexual morph differentiation in pea aphids ($P < 0.05$). Under the 14L:10D photoperiod, G3 consisted entirely of viviparae with no sexual morphs produced. Under the three short-day regimes (12L:12D, 10L:14D, and 8L:16D), sexual morphs were produced in all cases, with oviparae being exclusively wingless and males exclusively winged; no wingless males were observed. Among the three short-day treatments, the 12L:12D photoperiod yielded the highest male proportion (30.39%) and lowest ovipara proportion (55.67%). Under 8L:16D, no males were produced and oviparae reached their highest proportion (90.76%). Under 10L:14D, the proportion of viviparae was significantly higher while sexual morphs were significantly lower compared to the other two short-day treatments ($P < 0.05$). These results indicate that relatively longer day lengths favor male production, while shorter day lengths promote higher proportions of oviparae.

2.2 Effects of G1 Reproductive Period on Sexual Morph Differentiation

Table 2 shows that under the same photoperiod, the reproductive timing of G1 significantly affected morph proportions in G3, and morph proportions also differed among photoperiods at the same reproductive timing ($P < 0.05$). Under 12L:12D, G1 reproductive timing had no significant effect on vivipara or total sexual morph proportions ($P > 0.05$), but significantly affected male and ovipara proportions ($P < 0.05$). G1 individuals on day 2 of reproduction produced G3 offspring with the highest ovipara proportion and lowest male proportion, which differed significantly from those produced on days 7 and 12. Under 10L:14D, G1 reproductive timing significantly affected all morph proportions ($P < 0.05$). As G1 reproductive age increased, male proportion in G3 decreased while ovipara proportion increased, but G1 individuals on day 7 produced G3 offspring with the highest vivipara proportion and lowest sexual morph proportion. Under 8L:16D, no males were produced in G3, and G1 reproductive timing significantly affected both vivipara and ovipara proportions, thereby influencing the total sexual morph proportion ($P < 0.05$). On day 12 of G1 reproduction, G3 had the lowest vivipara proportion and highest ovipara proportion. These findings demonstrate that the duration of photoperiodic exposure in parental generations influences sexual morph differentiation in offspring.

2.3 Temporal Sequence of Sexual Morph Differentiation

Observations revealed that at 15°C, the total reproductive period of pea aphids was approximately 20 days. As shown in Figure 1 [Figure 1: see original paper], reproductive mode and sexual morph differentiation sequence were closely related to photoperiod, with morph proportions in G3 varying across the reproductive period of G2. Under 14L:10D, G3 consisted entirely of viviparae throughout the G2 reproductive period, with no sexual morphs produced, indicating exclusively parthenogenetic reproduction (Figure 1d). When photoperiod was shortened, reproduction shifted primarily to sexual mode with some parthenogenetic reproduction (Figures 1a, b, c). Under the three short-day regimes, oviparae dominated G3 offspring during the first 10 days of G2 reproduction, while males appeared after day 10, with the timing of male appearance becoming earlier as photoperiod shortened. Under 8L:16D, all offspring produced on day 1 were oviparae, and viviparae began to appear after day 5. Under 10L:14D and 12L:12D, males appeared on day 10 after initial production of oviparae and viviparae. These patterns demonstrate a distinct temporal sequence in sexual morph differentiation: oviparae appear first, followed by males, with viviparae being produced during the transition from oviparae to males.

Discussion and Conclusion

In nature, temperature and photoperiod act as critical environmental factors that coordinately influence organismal development, reproduction, and

distribution. Insect life cycles, diapause, generational alternation, and seasonal polyphenism in aphids are all closely associated with temperature and photoperiod variations [23]. In temperate regions, photoperiodic induction is the primary cause of insect diapause. Because photoperiod differences among geographic latitudes are constant, the critical photoperiod for diapause induction exhibits geographic variation within species [24-25]. Studies on different geographic populations of soybean aphid [17], cotton aphid [26], and Asian corn borer (*Ostrinia furnacalis*) [27] have confirmed that for short-day diapause insects, the critical photoperiod for diapause induction typically lengthens with increasing latitude. Previous reports indicate that at 20°C, pea aphids in Manitoba, Canada, produce sexual morphs when day length falls below 15 hours, with maximum male production under 15L:9D [13]. Lees [12] found that for a pea aphid population in Berkshire, England, sexual morphs appeared at 15°C under 14L:10D, which was most favorable for male production. Our results show that at 15°C, Lanzhou pea aphids produced no sexual morphs under 14L:10D, but began sexual morph differentiation under 12L:12D with maximum male production (30.39%). This discrepancy may be related to Lanzhou's lower latitude [28]. Under all three short-day treatments, sexual morph proportions exceeded 70%, indicating that the critical day length for sexual morph production in the Lanzhou population lies between 12 and 14 hours.

We found that oviparae in the Lanzhou population were exclusively wingless, while males were exclusively winged. Within the 8–12 hour day length range, male numbers decreased progressively as photoperiod shortened, while ovipara numbers increased. The temporal sequence of sexual morph differentiation—oviparae first, followed by males, with viviparae appearing during the transition—aligns with findings from international studies on pea aphids [12-13,29].

Previous research on winged versus wingless aphids has shown that under identical conditions, winged morphs have longer development times and require more nutrients because wing development represents an additional investment that necessitates extended development to acquire sufficient resources [29-31]. However, under natural conditions when environments become unfavorable (shortening photoperiod, food scarcity), aphids may adjust offspring morph ratios to maximize fitness returns from limited resources. For example, under 12L:12D, oviparae comprised 55.67% and males 30.39% of offspring, whereas under 8L:16D, oviparae reached 90.76% with no male production. As photoperiod shortened, increasing ovipara numbers would result in more overwintering eggs distributed across a wider area. Conversely, decreasing male numbers under shorter photoperiods may reflect either that sufficient males for mating were already produced during earlier, relatively longer day conditions, or that parental investment in males decreased because males appear later in the reproductive period and require longer development [17,29]. Additionally, the continued production of 10–25% viviparae during late reproduction under short-day conditions suggests that pea aphids can resume parthenogenetic reproduction and resume pest pressure when conditions become favorable again.

Thus, the observed patterns of sexual morph differentiation reflect maternal adjustment of offspring sex allocation in response to environmental changes and differences among sexual morphs.

We also found that G1 reproductive timing significantly affected morph proportions in G3 offspring, with significant differences among photoperiods at the same reproductive timing ($P < 0.05$). Under 12L:12D, male proportions in offspring tended to increase with G1 reproductive age, but this trend reversed under shorter photoperiods, with male proportions decreasing as G1 age increased, eventually reaching zero under the shortest photoperiod. Under 10L:14D and 8L:16D, ovipara proportions increased while vivipara proportions decreased with maternal age. The period around day 7 of G1 reproduction marked a significant transition point for sexual morph differentiation, consistent with findings for cotton and soybean aphids [15,17,26]. These maternal effects likely arise because environmental conditions (light, temperature, nutrition) experienced by mothers influence offspring phenotypes [32]. Guo et al. [33] reported that maternal photoperiod experience affects diapause incidence in *Cotesia plutellae*. Similarly, the reproductive timing of G1 pea aphids reflects the duration of photoperiodic exposure, thereby influencing sexual morph differentiation in offspring.

This study demonstrates that the critical photoperiod for sexual morph production in the green pea aphid in Lanzhou ranges from 12 to 14 hours, with sexual morph proportions varying among photoperiods and being influenced by parental reproductive timing. Relatively longer day lengths favor male induction, while shorter day lengths promote ovipara production. Sexual morph differentiation follows a distinct temporal sequence: oviparae appear first, followed by males, with a few viviparae produced during the transition. However, this study focused only on the green form. Since pea aphids have both red and green forms that exhibit significant differences in development and reproduction under identical host, temperature, and photoperiod conditions [19,21,34], whether the red form follows the same differentiation patterns requires further investigation. Additionally, because temperature and photoperiod act synergistically in nature, the interactive effects of these factors on sexual morph induction in pea aphids warrant further study.

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