Occurrence of Parthenogenesis in Potato Tuber Moth

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Abstract

Parthenogenesis, a natural form of asexual reproduction produced from unfertilized eggs, occurs in many insects in Hemiptera and Hymenoptera, but very rarely in Lepidoptera. The current study aimed to test the larval density dependent occurrence of parthenogenesis in potato tuber moth, Phthorimaea operculella (Zeller; Lepidoptera: Gelechiidae) under laboratory conditions. More than 10% of females out of 25 tested females that developed from the high larval density treatment at 45 larvae per tuber were capable to reproduce asexually. Both male and female offspring were produced parthenogenetically. The sexually reproductive offspring of a laboratory parthenogenetic population had a lower egg hatch rate, shorter larval stage, and shorter male life span when compared with the non-parthenogenetic population. This suggests that the sexually reproductive offspring of parthenogenetic population have a decreased overall fitness compared to the sexually reproductive offspring of non-parthenogenetic population.

Key words: Gelechiidae, larval density, asexual reproduction, biological parameter

Materials and Methods

Insect Source

The colony of P. operculella was established from larvae collected from a potato field located in Qujing, Yunnan Province (103.79 E°, 25.38 N°). The colony was managed in storage warehouses. Infestations in leaves and tubers often result in serious economic damages in the field. High populations of P. operculella may cause up to 70% losses in poorly managed storage warehouses (Rondon 2010). It is always impractical to control larvae that concealed in the tubers effectively by insecticide application (Cameron et al. 2002). More effective and environmentally-friendly control tactics are needed. Phenome traps have been used for population monitoring, mating disruption, and attract-and-kill of male moths (Hindenlang et al. 1975, Persoons et al. 1976, Kroschel and Zegarra 2010, Kroschel and Zegarra 2013). However, such practices can only be successful for insect species with sexual reproduction of which parthenogenesis development from unfertilized eggs does not occur. Therefore, it is crucial to evaluate whether potato tuber moth can reproduce parthenogenetically under certain conditions.

Parthenogenesis, a natural form of asexual reproduction in which offspring are produced from unfertilized eggs, occurs in many insects including aphids, bees, wasps, and beetles (Goudie and Oldroyd 2014, Ogawa and Miura 2014, Ma and Schwander 2017). This type of reproduction also occurs in several families of Lepidoptera, for example, fall cankerworm, Alsophila pometaria (Harris; Lepidoptera: Geometridae) (Mitter and Futuyma 1977), leafminer moth Phyllonorycter emberizaepenella (Bouché; Lepidoptera: Gracillariidae) (Mozurrattis et al. 2002), and tomato leafminer, Tuta absoluta (Meyrick; Lepidoptera: Gelechiidae) (Megido et al. 2012).

The biology of P. operculella has been well documented (Fenemore 1977, Foot 1979, Dogramaci et al. 2010, Rondon 2010, Kroschel and Zegarra 2013), but parthenogenesis in this species has sparsely been mentioned. Early reports noted that parthenogenetic reproduction did occur in laboratory population of P. operculella but very rarely (Poos and Peters 1927, Hofmaster 1949). Several other reports indicated that no parthenogenesis has ever been observed (Fenemore 1977, Nabi and Harrison 1983). To date, it remains questionable whether parthenogenesis occurs in P. operculella. The current study aimed to investigate 1) conditions (larval density) to induce parthenogenesis in the laboratory population of P. operculella and 2) the major biological parameters of the parthenogenetic and non-parthenogenetic (normal) populations under laboratory conditions.
25.51 N°) in April 2016. The infested tubers were held in a mesh cage (L × W × H = 35 × 35 × 35 cm) in which sand was provided as a pupation medium to allow easy harvesting of pupae. The cages were placed in an insect-rearing room under 24 ± 2°C, 70 ± 5% RH and 14:10 (L:D) h photoperiod (Gui and Li 2003). The pupae were removed from sand and kept individually in glass tubes (2 cm in diameter and 4 cm high) under the same conditions. After emergence, twenty adult pairs (opposite sex) were confined in a plastic cylindrical container (13 cm in diameter and 14.5 cm high) for copulation and oviposition, and 10% honey water was provided through a daily refreshed cotton ball. The open top of the plastic container was covered with a single layer of cotton gauze over a piece of filter paper for egg laying. The filter paper was changed daily to collect eggs to obtain newly hatched larvae for continuous rearing and/or testing. The larvae were transferred to insect-free potato tubers (average weight 130 ± 2 g) at a density of 15 larvae per tuber. The potato tubers were punctured with a needle to facilitate larval infestation prior to introduction of larvae. About 30 such infested tubers were placed in a mesh cage with same dimension as above and kept under the same environmental conditions as mentioned above till pupation and adult emergence. A sexually reproductive population was achieved at density of 15 larvae per tuber using this process.

Parthenogenesis Test
Newly hatched larvae from the sexually reproductive population were transferred to insect-free potato tubers (average weight 130 ± 2 g) at the density levels of 5, 25, and 45 larvae per tuber. Twenty to forty tubers were prepared for each density to obtain enough insects (>100 females) for testing. The infested tubers of a given density were held together in a mesh cage until pupation. The F1–generation pupae were individually transferred into glass tubes (2 cm in diameter and 4 cm high) and sexed after emergence. The newly emerged virgin females (F1–generation) were placed individually for a week in plastic cups (=220 ml) containing a fresh insect-free potato tuber and 10% honey water. The open top of the cup was sealed with a single layer of cotton gauze. The cups were held together in a mesh cage and kept in the insect-rearing room as described above. A group of 25 virgin females (F1–generation) from the same density (5, 25 or 45 larvae per tuber) treatment were tested as a replication. The virgin females laid eggs (F1–generation) on the fresh potato tuber. Successful egg hatch (F1–generation) confirmed by the presence of feces excreted by neonate larvae that fed on the tuber. The tuber with the F1 larvae was transferred to a new cage until pupation. Then, the pupae were individually transferred into glass tubes and sexed after emergence, indicating occurrence of parthenogenesis. The test was replicated four times.

Biological Parameters of Non-parthenogenetic and Parthenogenetic Offspring
About 200 potato tubers were infested at the density of 45 larvae per tuber to obtain parthenogenetic F1–generation females, and 20 tubers were infested at the density of 15 larvae per tuber to obtain non-parthenogenetic F1–generation females. Biological parameters of sexually reproduced offspring (F1–generation) from non-parthenogenetic population (CK) and parthenogenetic individuals (Treatment) were derived from the following assays: Parthenogenetically reproduced larvae (F1–generation) were reared on insect-free potato tubers at the density of 15 larvae per tuber to obtain the F2–generation adults of the parthenogenetic offspring. Ten pairs (opposite sex) of the newly emerged F1 adults were placed in a plastic cylindrical container (13 cm in diameter and 14.5 cm high), and provided with 10% honey water. Mating rate of F1 females was determined by dissecting each female 1 d after death and checking for the presence of spermatophores in the bursa copulatrix. The lifespan of the F1–generation female and male adults was also recorded. The eggs (F1–generation) produced were collected every day and counted daily for the first 4 d. Egg hatch was observed under a microscope (× 10) at the fourth day after egg collection. At the same time, sexually reproduced larvae (F1–generation) from non-parthenogenetic population (F1–generation) were reared and observed in the same manner. There were four replicates for treatment and for control.

In addition, a portion of eggs of the F1 generation (both Treatment and CK) deposited on the second day were used for further observations. After hatching, 30 first-instar larvae were transferred to ‘punctured’ tubers at the density of 15 larvae per tuber, and reared under the same conditions as above until adults. Larval duration, larval survival rate, pupal weight, and sex ratio of F1–generation adults, and the number of eggs produced by F1–generation adults (F1–generation) were recorded. Sexually reproduced larvae from non-parthenogenetic population were reared and observed in the same manner. There were four replicates for treatment and for control.

Statistical Analysis
One-way ANOVA was performed for the influence of larval density in potato tubers on the percentage of female adults that reproduce parthenogenetically followed by Tukey’s test for mean separation. All other comparisons between treatments were analyzed using student’s t test at 5% level (SPSS17.0).

Results
Effect of Larva Density on Parthenogenesis Occurrence in P. operculella
Under laboratory conditions, no viable eggs were produced by virgin female moths from the treatment of five larvae per tuber, indicating that no parthenogenetic reproduction had occurred. When larval densities increased to 25 and 45 larvae per tuber, parthenogenesis occurred at rates of 6 to 12%, respectively. The rate of parthenogenesis occurrence increased numerically with the increase of larval density (ANOVA: F2,9 = 5.809, P = 0.024, Fig. 1). Furthermore, both male and female offspring were produced by parthenogenetic females. More females emerged at lower larval density (25 larvae per tuber), on the contrary, more males emerged at higher larval density (45 larvae per tuber), and no significant difference was observed.

![Fig. 1. Influence of larval density in potato tubers on percentage of female adults that reproduce parthenogenetically.](image-url)
between the number of females and males emerged at both densities tested \( (P > 0.05) \) (Fig. 2).

**Biological Parameters of Parthenogenetic Offspring**

During the first 4 d, mated females of the F₁ generation of the parthenogenetic population deposited equal numbers of eggs as the non-parthenogenetic population. Furthermore, there was no significant difference in egg hatch rate of the F₂ generation between the two populations except for the first day \( (P = 0.002, \text{Table 1}) \).

Larval stage duration of the sexually reproduced F₁ generation of the parthenogenetic population (6.3 ± 0.1 d) was significantly shorter than that of the non-parthenogenetic population (7.3 ± 0.3 d) \( (P = 0.004) \). No significant differences in larval survival rate and in both biological parameters of pupa were found between the two populations \( (P > 0.05, \text{Table 2}) \).

Only male adults of the sexually reproduced parthenogenetic offspring had a shorter life span \( (13.3 ± 0.3 \text{ d}) \) than that of the non-parthenogenetic population \( (15.4 ± 0.3 \text{ d}) \) \( (P < 0.001) \). For female adults of the F₂ generation, there were no significant differences in life span, egg production, and mating rate between the two populations \( (P > 0.05, \text{Table 2}) \).

**Discussion**

Previous studies have documented that parthenogenesis occurred in about 20 species in the Bombycidae, Gelechiidae, Gracillariidae, Lasiocampidae, Liparidae, Nepticulidae, Saturnidae, Sesiidae, Sphingidae of Lepidoptera \( (\text{Suomalainen 1962, Mitter and Futuyma 1977, Lynch 1984, Menken and Wieboschsteeman 1988, Mozūraitis et al. 2002, Megido et al. 2012}) \). The results of this study revealed that parthenogenesis did occur under laboratory conditions in *P. operculella*.

Parthenogenesis can produce offspring of both sexes (deuterotoky), only females (thelytoky, e.g., aphids), or only males (arrhenotoky, e.g., most wasps) \( (\text{Stouthamer et al. 1990, Pannebakker et al. 2004}) \). The fact that unfertilized eggs developed into both males and females indicated a deuterotoky type of parthenogenesis in *P. operculella*. In addition, no significant difference in mating rate between the parthenogenetic and non-parthenogenetic (sexually) reproduced offspring \( (\text{Table 2}) \) suggests that parthenogenesis has no negative impact on mating capability of parthenogenetic offspring. Ma et al. \( (2010) \) reported that more males were produced when larvae were reared at higher density in potato tubers. This situation would render females a higher chance to encounter males for copulation. Thus, we speculate that the parthenogenesis in *P. operculella* is extremely

![Fig. 2. Number of adults produced by parthenogenetic females of potato tuber moth, *P. operculella*.](image)

**Table 1.** Number of eggs (mean ± SE) produced by a group of 10 females of the F₁ generation and egg hatch rate (mean ± SE) \( (n = 4) \)

| Reproduction mode | First day | Second day | Third day | Fourth day |
|-------------------|-----------|------------|-----------|------------|
|                   | No. of egg| Hatch (%)  | No. of egg| Hatch (%)  |
|                   |           |            |           |            |
| Parthenogenesis    | 557.5 ± 17.2| 69.4 ± 2.1*| 548 ± 32.6| 61.9 ± 4.7 |
| Non-parthenogenesis| 660.5 ± 39.8| 82.3 ± 1.5 | 543 ± 34.9| 66.8 ± 2.5 |

*Mean significant differences between parthenogenesis treatment and non-parthenogenesis control at 0.05 level.

**Table 2.** Biological parameters of F₂ generation

| Biological parameters | Parthenogenesis | Non-parthenogenesis |
|-----------------------|-----------------|---------------------|
| Larvae                |                 |                     |
| Duration (d)          | 6.3 ± 0.1*      | 7.3 ± 0.3           |
| Survival rate (%)     | 98.4 ± 1.6      | 99.2 ± 0.8          |
| Pupae                 |                 |                     |
| Duration (d)          | Female          | 8.1 ± 0.1           | 7.7 ± 0.1  |
|                       | Male            | 7.9 ± 0.1           | 7.8 ± 0.1  |
| Weight (mg)           | Female          | 8.7 ± 0.3           | 8.6 ± 0.2  |
|                       | Male            | 4.6 ± 0.2           | 4.2 ± 0.1  |
| Adults                |                 |                     |
| Sex ratio (%)         | Female          | 52.6 ± 3.2          | 47.6 ± 3.2 |
|                       | Male            | 47.4 ± 3.2          | 52.4 ± 3.2 |
| Duration (d)          | Female          | 12.7 ± 0.5          | 12.5 ± 0.5 |
|                       | Male            | 13.3 ± 0.4*         | 15.4 ± 0.3 |
| Mating rate (%)       | 85.0 ± 2.9      | 87.5 ± 2.5          |
| Number of eggs        | 175.3 ± 2.5     | 173.1 ± 4.2         |

Pupae weight was obtained by subtracting the weight of the cocoon shell by the weight of the full cocoon. Pupae were held individually and sexed after emergence. Number of eggs means the average number of eggs per 10 females in the first 4 d.

*Significant difference between parthenogenesis treatment and non-parthenogenesis control at 0.05 level.
Previous reports have found that food availability was an important factor to transition of the reproductive mode of some insect species (Scheu and Drossel 2007, Wehner et al. 2014). Parthenogenesis generally occurs when food resources are available in excess, while sexuality correlates with food shortage. However, food shortage or a nutrition deficiency experienced at larval stage affects adult morphology and life-history traits, especially in species where adults do not feed (Nevo and Coll 2001, Jones et al. 2011, Bhavanam and Trewick 2017). In present study, we found that high larval density triggered parthenogenetic reproduction in females of P. operculella, possibly because of an emergency response to adverse overcrowding conditions. From an adaptive perspective, one advantage of this asexual reproduction was to maintain populations at a certain level in adverse conditions or in the absence of males (Abbes and Chermiti 2014). The results of the sexually reproductive offspring of the parthenogenetic population had a lower egg hatch rate, higher larval mortality, shorter larval duration, shorter life span, and lower sex ratio than that of non-parthenogenesis (normal) population suggests that malnutrition caused by overcrowding during larval development negatively impacted on transgenerational reproduction (Fischer and Fiedler 2001, Bauerfeind and Fischer 2005, Bhavanam et al. 2012). Thus, the occurrence of parthenogenesis under less favorable conditions could reduce reproductive potential and be beneficial to the overall fitness of the population under stressful conditions. We found a relatively low occurrence rate (<12%) of parthenogenesis in P. operculella even at the highest larval density tested. P. operculella lay eggs singly or in groups of 3 to 15 near the eye buds of potato tubers under storage conditions (Rondon 2010). The possibility of the occurrence of parthenogenesis in large wild populations under field or storage warehouse conditions should be investigated (Hofmaster 1949). It seems likely for natural populations of P. operculella in heavily infested potato field but not in storage houses under controlled temperature and humidity to reproduce parthenogenetically. Whether such a low rate of parthenogenesis would significantly affect the effectiveness of any male related control tactics also requires further investigation.

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References Cited
Abbes, K., and B. Chermiti. 2014. Propensity of three Tunisian populations of the tomato leafminer Tuta absoluta (Lepidoptera: Gelechiidae) for deuterotokous parthenogenetic reproduction. Afr. Entomol. 22: 538–544.
Andreadis, S. S., C. G. Spanoudis, G. Zakka, B. Aslanidou, S. Noukari, and M. Savopoulou-Soukalni. 2017. Effect of temperature on rate of development, survival and adult longevity of Phthorimaea operculaella (Lepidoptera: Gelechiidae). Eur. J. Entomol. 114: 35–41.
Anfora, G., S. Vitagliano, M. C. Larsson, P. Witzgall, M. Tasin, G. S. Germinara, and A. De Cristofaro. 2014. Disruption of Phthorimaea operculaella (Lepidoptera: Gelechiidae) oviposition by the application of host plant volatiles. Pest Manag, Sci. 70: 628–635.
Bauerfeind, S. S., and K. Fischer. 2005. Effects of food stress and density in different life stages on reproduction in a butterfly. Oikos 111: 514–524.
Bhavanam, S., and S. Trewick. 2017. Effects of larval crowding and nutrient limitation on male phenotype, reproductive investment and strategy in Ephestia kuehniella Zeller (Insecta: Lepidoptera). J. Stored Prod. Res., 71: 64–71.
Bhavanam, S. P., Q. Wang, and X. Z. He. 2012. Effect of nutritional stress and larval crowding on survival, development and reproductive output of Mediterranean flour moth, Ephestia kuehniella Zeller. N. Z. Plant Prot. 65: 138–141.
Cameron, P. J., G. P. Walker, G. M. Penny, and P. J. Wigley. 2002. Movement of potato tuberworm (Lepidoptera: Gelechiidae) within and between crops, and some comparisons with diamondback moth (Lepidoptera: Plutellidae). Environ. Entomol. 31: 65–75.
Dogramaci, M., S. I. Rondon, and S. J. Debano. 2010. The effect of soil depth and exposure to winter conditions on survival of the potato tuberworm, Phthorimaea operculaella. Entomol. Exp. Appl. 129: 332–339.
Fenemore, P. G. 1977. Oviposition of potato tuber moth, Phthorimaea operculaella Zell. (Lepidoptera: Gelechiidae); fecundity in relation to mated state, age, and pupal weight. N. Z. J. Zool. 4: 187–191.
Fischer, K., and K. Fiedler. 2001. Effects of larval starvation on adult life-history traits in the butterfly species Lycaena tityrus (Lepidoptera: Lycaenidae). Entomol. Gen. 25: 249–254.
Foot, M. A. 1979. Bionomics of the potato tuber moth, Phthorimaea operculaella (Lepidoptera: Gelechiidae), at Pukekohe. N. Z. J. Zool. 6: 623–636.
Goudie, F., and B. P. Oldroyd. 2014. Thelytoky in the honey bee. Apidologie 45: 306–326.
Gu, F.-R., and Z.-Y. Li. 2003. A method for rearing the potato tuber moth, Phthorimaea operculaella on potato. Entomol. Knowlegd. 40: 187–189.
Hindenlang, D. M., J. R. McLaughlin, R. M. Guiliano, and L. B. Hendry. 1975. A sex pheromone in the potato tuberworm moth, Phthorimaea operculaella (Zeller): biological assay and preliminary chemical investigation. J. Chem. Ecol. 1: 465–473.
Hofmaster, R. N. 1949. Biology and control of the potato tuberworm with special reference to eastern Virginia. Virginia Truck Experiment Station Bulletin 111: 1826–1882.
Jones, R. T., A. Bressan, A. M. Greenwell, and N. Fierer. 2011. Bacterial communities of two parthenogenetic aphid species cocolonizing two host plants across the Hawaiian Islands. Appl. Environ. Microbiol. 77: 8345–8349.
Kroschel, J., and O. Zaggar. 2010. Attract-and-kill: a new strategy for the management of the potato tuber moths Phthorimaea operculaella (Zeller) and Symmetrismicha tangolias (Gyen) in potato: laboratory experiments towards optimising pheromone and insecticide concentration. Pest Manag, Sci. 66: 490–496.
Kroschel, J., and O. Zaggar. 2013. Attract-and-kill as a new strategy for the management of the potato tuber moths Phthorimaea operculaella (Zeller) and Symmetrismicha tangolias (Gyen) in potato: evaluation of its efficacy under potato field and storage conditions. Pest Manag. Sci. 69: 1205–1215.
Lynch, M. 1984. Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. Q. Rev. Biol. 59: 257–290.
Ma, W. J., and T. Schwander. 2017. Patterns and mechanisms in instances of endosymbiont-induced parthenogenesis. J. Evol. Biol. 30: 868–888.
Ma, Y. F., Y. Xu, N. Li, Z. Y. Li, Y. Q. He, and C. Xiao. 2010. Effect of larval density on growth, development and reproduction of potato tuber moth, Phthorimaea operculaella. Chin. Bull. Entomol. 47: 694–699.
Megido, R. C., E. Haubruege, and F. J. Verhagen. 2012. First evidence of deuterotokous parthenogenesis in the tomato leafminer, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae). J. Pest Sci. 85: 409–412.
Menken, S., and M. Wiebschotchseaman. 1988. Clonal diversity, population structure, and dispersal in the parthenogenetic moth Ectoedemia argyropoeza. Entomol. Exp. Appl. 49: 141–152.
Mitter, C., and D. Futuyma. 1977. Parthenogenesis in the fall cankerworm, Alsophila pometaria (Lepidoptera, Geometridae). Entomol. Exp. Appl. 21: 192–198.
Mozuraitis, R., V. Buida, I. Llibikas, C. R. Unelius, and A. K. Borg-Karlson. 2002. Parthenogenesis, calling behavior, and insect-released volatiles of leafminer moth Phyllonorycter erberzaegenteliana. J. Chem. Ecol. 28: 1191–1208.
Nabi, M. N., and R. A. Harrison. 1983. Activity of sperm and fertility in the potato moth, Phthorimaea operculaella. J. Insect Physiol. 29: 431–435.
Nevo, E., and M. Coll. 2001. Effect of nitrogen fertilization on Aphis gossypii (Homoptera: Aphididae): variation in size, color, and reproduction. J. Econ. Entomol. 94: 27–32.
Ogawa, K., and T. Miura. 2014. Aphid polyphenisms: trans-generational developmental regulation through viviparity. Front. Physiol. 5: 1.
Pannebakker, B. A., L. P. Pijnacker, B. J. Zwaan, and L. W. Beukeboom. 2004. Cytology of Wolbachia-induced parthenogenesis in *Leptopilina clavipes* (Hymenoptera: Figitidae). Genome. 47: 299–303.

Persoons, C. J., S. Voerman, P. E. J. Verwiel, F. J. Ritter, W. J. Nooyen, and A. K. Minks. 1976. Sex pheromone of the potato tuberworm moth, *Phthorimaea operculella*, identification and field evaluation. Entomol. Exp. Appl. 20: 289–300.

Poos, F. W., and H. S. Peters. 1927. The potato tuber-worm. Va. Truck Exp. Stn. Bull. 61: 596–630.

Rondon, S. I. 2010. The potato tuberworm: a literature review of its biology, ecology, and control. Am. J. Potato Res. 87: 149–166.

Scheu, S., and B. Drossel. 2007. Sexual reproduction prevails in a world of structured resources in short supply. Proc. Biol. Sci. 274: 1225–1231.

Stouthamer, R., R. F. Luck, and W. D. Hamilton. 1990. Antibiotics cause parthenogenetic Trichogramma (Hymenoptera/Trichogrammatidae) to revert to sex. Proc. Natl. Acad. Sci. U. S. A. 87: 2424–2427.

Suomalainen, E. 1962. Significance of parthenogenesis in the evolution of insects. Annu. Rev. Entomol. 7: 349–366.

Wehner, K., S. Scheu, and M. Maraun. 2014. Resource availability as driving factor of the reproductive mode in soil microarthropods (Acari, Oribatida). Plos One. 9: e104243.