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The Quantitative Genetics of Prescutellar Bristles in Melon Fly, *Bactrocera cucurbitae* (Coquillett)

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Abstract

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The polymorphism of prescutellar bristles (*prsc*) in melon fly, *Bactrocera cucurbitae* (Coquillett), was investigated by artificial selection of phenotypes and quantitative genetic analysis. In the wild strain, generally more than 95% population have 2 *prsc* while some may have 4 *prsc*. By selection, however, a group of phenotypes was appeared with the number of *prsc* reach up to 14 and more. All multiplied bristles were randomly arranged in a region of posterior half of *scutum* and between the lateral *vitta*. The quantitative genetic analysis was conducted by crossing two strains of different phenotypes, one from each strain. In each cross only one virgin male and one virgin female were paired, with a total of 6 pairs in each experiment, and 200–500 offsprings from each experiment were examined for their phenotypes. For each cross, only one 2-*prsc* individuals (the major component of the wild population) and one 12-*prsc* or 14-*prsc* individuals from the selected strains were interbred, and then inbred F_1 for the F_2 progeny. The results showed that for 2-*prsc* × 12-*prsc*, the F_1 progeny constituted a group of phenotypes with different *prsc* numbers, and the mean was 4.7 ± 0.9 which was in-between two parental phenotypes. The mean of *prsc* in F_2 is 4.7 ± 1.9. The difference between the means of F_1 and F_2 is insignificant in the *t*-test ($P > 0.05$). For 2-*prsc* × 14-*prsc*, the F_1 progeny constituted a group of phenotypes, with the mean of 5.0 ± 1.0 which was in-between their parental phenotypes. The F_2 offsprings had their mean at 5.1 ± 2.3. The *t*-test indicated no significant difference between the means of F_1 and F_2 ($P > 0.05$). Moreover, as the coefficients of variation of F_2 were higher than those of F_1 (40.1% > 19.5% versus 45.9% > 21.7%), it suggests that more variability around the mean of F_2 than that of F_1. The extreme values in *prsc* for F_2 were extended to the range of their parental values (2–15 *prsc* and 1–17 *prsc*, respectively) than did the extreme values of F_1. Results of this study were similar to that reported by Emerson & East (1913), indicating that the *prsc* of melon fly is a continuous and quantitative trait in inheritance.

Key words: *Bactrocera cucurbitae*, Prescutellar bristles, Quantitative genetic analysis, Quantitative trait.

INTRODUCTION

The melon fly, *Bactrocera cucurbitae* (Coquillett), is an economic important pest in agriculture, and identifying this tephritidae species is a routine practice in the integrated control program. The thorax bristles are important morphological characters in the fruit fly identification. For example, the appearance of scutellar bristles in thorax has been suggested by Drew & Hancock (2001) in an attempt to determine which characters are primitive within the Da-

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cini. White (2001) described that the prescutellar bristles (*prsc*) can be used as one of the characters in classifying subgenera of *Bactrocera* and *Dacus*. Particularly, for the adult identification of melon fly, White & Elson-Harris (1992) described that a pair of *prsc* and 2 or 4 (rarely) scutellar bristles were present.

In 1996, a field survey of melon fly in Taiwan revealed that the majority possesses a pair of *prsc* as that reported by White & Elson-Harris (1992). However, there were 5% of the population otherwise, and 0 to 4 *prsc* other than 2 *prsc* were found in them. This *prsc* polymorphism had caused confusion in the specimen identification and demanded further clarification. The insect bristles are external sensory organs of the peripheral nervous system. Any change in their number will involve the basic innervation difference in the gene controlled embryonic development. For example, in *Drosophila*, the number of bristles has been a classic quantitative genetic character (Mackey 1995), and the development of bristles is associated with the expression of genes of the achaete-scute complex (ASC) in the epidermis of *Drosophila* (Brakefield *et al*. 2003). By artificially selected the numbers of sternopleural and abdominal bristle in the laboratory established wild Drosophila strain, the marker genotypes were analyzed by quantitative trait loci (QTL) mapping techniques for their phenotype (Long *et al*. 1995; Lynch & Walsh 1998; Gurganus *et al*. 1999). Do the melon flies have the *prsc* polymorphism? How is it controlled genetically? Both are the purposes of this study.

MATERIALS AND METHODS

The caged melon flies were reared at room temperature, on a diet of agar water gel, protein hydrolyze and sugar. The fresh cucumber was used for egg laying. The morphology of melon fly was observed under a dissecting microscope. The intra-alar bristles were not included in the *prsc* count (Appendix). The live specimens were either immobilized by low temperature or restrained inside a glass vial for *prsc* phenotype inspection. The morphological description and nomenclature of Tephritidae by Drew & Hancock (1994) was adopted in this study.

The laboratory reared wild (LRW) strain

A LRW-strain was established by harvesting the larvae from field infested cucumbers and reared in the laboratory.

The artificial selected strains

Strains with different *prsc* numbers were selected artificially. In the selection, the melon flies were harvested at pupal stage, and each pupa was transferred into a glass vial. Every day, the freshly emerged flies were inspected for sexes and phenotype of *prsc* before being selected for pairing. The successive selection was conducted by pairing the desired phenotype from generation to generation, and each selection consisted of six pairs.

Characterizing the strains in regard of *prsc*

For each strain around 500 to 1000 flies of each selected generation were inspected for their *prsc* numbers. The *prsc*-phenotypes appeared in a continuously distributed trait, the mean, the standard deviation and the range of *prsc* were used to define the character of a strain.

Genetic studies

To be precise in the genetic study, only one desired phenotype from each strain was selected for crossing. The genetic study was carried out by crossing two different phenotypes from two different strains, and then inbred F_1 for the F_2 progeny. All experiments consisted of six pairs, and 200 to 500 offsprings from each replicate were examined for the *prsc* number count.

Statistical analysis

The *F*-test and the *t*-test were used to vali-

date the significance of difference between any two strains. All *prsc* results were separately recorded according to male and female.

RESULTS

The strains and the selection results

The LRW strain has five phenotypes. The majority (96.1%) of the population has 2 *prsc*, while 0.2% had no *prsc*, 1.0% had one *prsc*, 2.4% had 3 *prsc* and 0.3% had 4 *prsc* (Fig. 1A). The mean value of the LRW strain was 2.0 ± 0.2 in the range of 0–4 *prsc*. The phenotype constitute of this strain was frequently examined to assure that the *prsc* character of wild strain was maintained.

The artificial selection was to establish the mono-phenotype strains of both the 2-*prsc* and 4-*prsc* phenotypes.

The 2-*prsc* strain

After one hundred generations, the mono-phenotype strain could not be selected. The 2-*prsc* phenotype can only be improved from 96.1% to 97.3%, and the difference from the LRW-strain is not significant ($P > 0.05$), though both the 0- and the 4-*prsc* phenotypes were eliminated, and only the 1- to 3-*prsc* phenotypes remained at 1% and 1.6%, respectively (Fig. 1B). The mean value of the 2-*prsc* strain was 2.0 ± 0.2 in the range of 1–3 *prsc*.

The 4-*prsc* strain

The melon fly responded to the 4-*prsc* selection rapidly (Fig. 2). The 2-*prsc* phenotype decreased from 95% to less than 5% within 3 generations, and further lowered to less than 0.1% in 10 generations. The 4-*prsc* phenotype increased from 0.1% to 70% in 3 generations, and then reached a steady state between 75% and 85% of the population. The population hosts a continuous distributed trait of *prsc* phenotypes. At the end of 90 generations of selection, the 1-, the 2-, the 3-, the 4-, the 5-, the 6-, and the 7-*prsc* phenotypes were 0.1, 0.6, 5.5, 84.7, 9.3, 0.4, and 0.1% of the population,

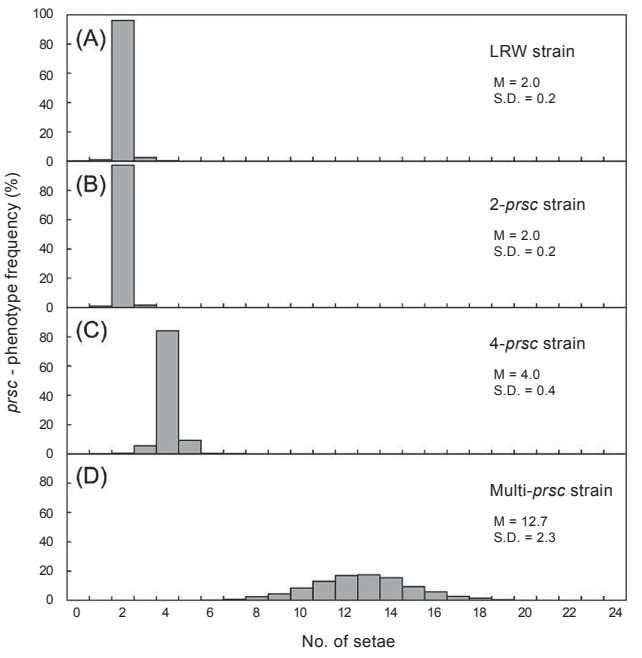


Fig. 1. The prsc-phenotype distribution of (A) the laboratory reared wild strain (LRW); (B) the selected 2-*prsc* strain; (C) the selected 4-*prsc* strain; and (D) the selected multiple-*prsc* strain. *prsc*: prescutellar bristles.

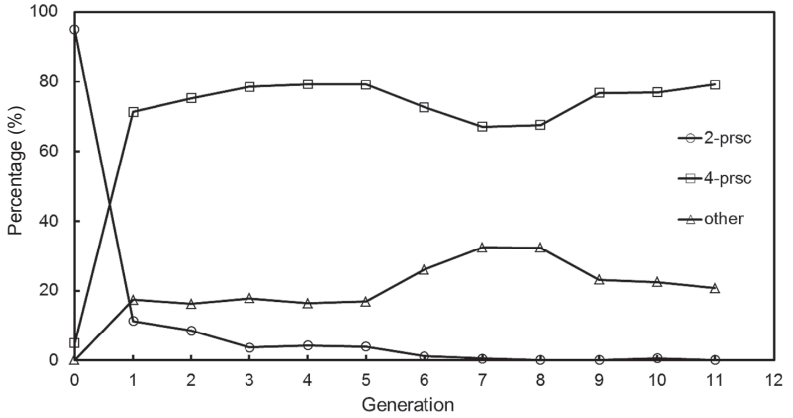


Fig. 2. The frequency variation of different *prsc*-phenotypes in the first ten generations selection of the 4-*prsc* strain. *prsc*: prescutellar bristles.

respectively (Fig. 1C). The mean value of the 4-*prsc* strain was 4.0 ± 0.4 in the range of 1–7 *prsc*.

Both the 2-*prsc* and the 4-*prsc* strain selections proclaimed that it was not possible to select a strain with only one phenotype or the mono-phenotype. Since more phenotypes appeared in the selection, it led to a decision of selecting a strain with more *prsc* or the multiple-*prsc* strain.

The multiple-*prsc* strain

The selection of multiple-*prsc* strain was preceded by successively pairing the individuals with the highest number of *prsc* from generation to generation. And after 40 generations, a colony of melon flies with multiple *prsc* in continuous traits was selected (Fig. 1D). The highest frequency is 17.5% for the 13-*prsc* phenotype, while the 12- and the 14-*prsc* phenotypes were 17.0 and 15.6%, respectively. For the multiple-*prsc* strain, the mean was 12.3 ± 1.9 in the range of 8–19 *prsc*.

The stability of the multiple-*prsc* strain was examined by lifting the selection for 10 generations, and the mean remained almost unchanged, i.e., mean = 12.7 ± 2.3 with the range of 5–23 *prsc*. The increase of variance is due to the range expansion of phenotypes. The stable result suggests that the strain is homozygous for the genes controlling *prsc*. The advantage to have the multiple-*prsc* strain is that it provides multiple choices of phenotypes for the genetic study.

In morphology, the *prsc* only multiplied in a region of the posterior half of *scutum* and between the *lateral postsutural vitte* (Plates A, B, C & D). The distribution of multiplied *prsc* in posterior *scutum* is symmetric but without a fixed pattern as the analysis of 600 flies of the multiple *prsc* strain in three repeats (Table 1). Simpson *et al*. (1999) suggested that the spatial distribution of sensory bristles on the notum of different species of *Diptera* is that the species displaying ancestral features have a simple organization of random distribution, which may also be applied to the primitive stage of bristle development in selected strains.

The result of artificial selection has demonstrated that the polymorphism of *prsc* is evident in the melon fly and cannot be eliminated by selection. All *prsc* test results showed that there is no difference between male and female, hence genetically there is no sex linkage involved in the *prsc* determination.

Genetic study

In *prsc* selections, the continuous trait in the form of a group of phenotypes always appeared, and the repeated phenomena implicate

Table 1. The left vs. right symmetric analysis of *prsc* (prescutellar bristles) distribution in the scutum of multiple-*prsc* strain melon flies.

Test no. (N = 200)	No. of *prsc* ± S.D.		*t*-value	*P*-value
	Left	Right		
1	6.3 ± 1.4	6.2 ± 1.4	0.085	0.93*
2	6.3 ± 1.5	6.4 ± 1.3	0.854	0.39
3	6.3 ± 1.5	6.3 ± 1.5	-0.331	0.74

* The differences of all three replicates are not significant at $P > 0.05$ level.

that the quantitative genetics is the possible control mechanism for *prsc* in melon fly. In order to search for more credible evidences, a classic example of quantitative genetic analysis of corn ear length by Emerson & East (1913) was simulated. By crossing two distinctly different phenotypes from two strains, both were stable and presumed to be homozygous for the genes controlling *prsc*. And then the interbred F_1 to the F_2 generation should provide the analytic evidences for quantitative genetics.

The first test is the cross between the 2-*prsc* and the 12-*prsc* phenotypes

The 2-*prsc* individuals from the 2-*prsc* strain and the 12-*prsc* individuals from the multiple-*prsc* strain were chosen for the cross. Fig. 3 presents the phenotypes of parents, F_1 progeny and the inbred F_2 progeny, respectively, in frequency histograms. The F_1 progeny constituted a group of phenotypes with *prsc* number ranged 2–8 and the mean of 4.7 ± 0.9 which was in-between two parental phenotypes. The mean of *prsc* in F_2 was 4.7 ± 1.9 and the range was 2–15 *prsc*. The difference between the means of F_1 and F_2 is insignificant in the *t*-test. The greater variance of F_2 than F_1 is due to the extreme value of F_2 phenotypes extended to two parental strains.

The 2nd test, the cross of the 2-*prsc* and the 14-*prsc* phenotypes

The 2-*prsc* individuals from the 2-*prsc* strain and the 14-*prsc* individuals from the

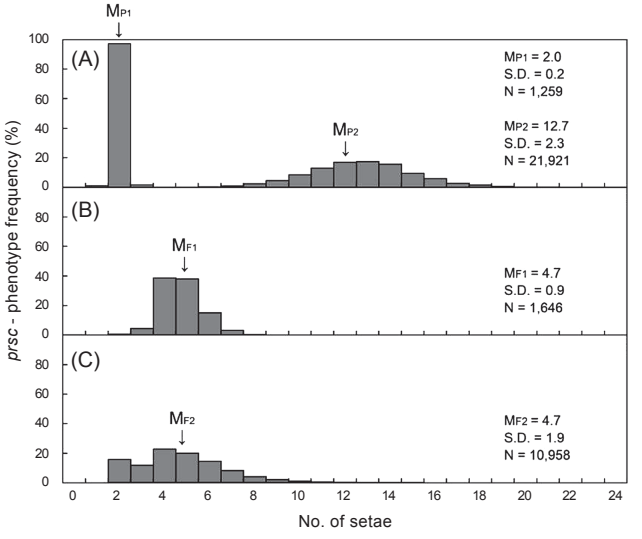


Fig. 3. Histogram of phenotypes frequencies with different numbers of *prsc* from (A) the parental strains, (B) the F_1 and (C) the F_2 generations in the 2-*prsc* × 12-*prsc* phenotypes cross test. *prsc*: prescutellar bristles.

multiple *prsc* strain were chosen for the cross. The Figs. 4A, B and C present the phenotypes of parents, F_1 progeny and the inbred F_2 progeny in frequency histograms. The F_1 progeny constituted a group of phenotypes ranged 1–9 *prsc* with the mean of 5.0 ± 1.0 which was in-between two parental phenotypes. The F_2 offsprings had the mean at 5.1 ± 2.3 and the range was 1–17 *prsc*. As the 1st test, the *t*-test concluded that the difference between the means of F_1 and F_2 is not significant. The greater variance of F_2 is due to the extreme value of phenotypes extended to two parental strains.

In both tests, results were similar to quantitative inheritance study reported by Russell (2005): (1) The mean value of the quantitative trait, or the *prsc* in the F_1 progeny was intermediate between the means of the two parental phenotypes. (2) The mean value of the *prsc* in the F_2 progeny was approximately equal to the mean for the F_1 population. (3) When standard derivation was compared, the F_2 showed more *prsc* variability around the mean than the F_1 did. (4) The extreme values for the *prsc* in the F_2 extended to the two parental values than did the extreme values of the F_1.

DISCUSSION

The polymorphism of thorax *prsc*-bristles is found in melon fly, and the 2-*prsc* phenotype is dominated at 95% in the wild population of Taiwan. The initial attempt is to select the mono-phenotype lines for the genetic study. However, it was the continuous trait of phenotypes appeared in the selection, a mono-phenotype strain was an in vain attempt. The effort was redirected to select melon fly with more *prsc* for the genetic investigation. Three strains were selected from the LRW strain, which itself is a strain with a narrow ray of phenotypes. The selection only moves a window, or the mean, along a continuous trait to a certain selected point. The strictness of selection enforced direct influences on the variation and the range of phenotypes in progeny. For example, when the 12-*rs* individuals were replaced by the 14-*prsc* individuals in the second genetic test, the mean of *prsc* in F_1 increased from 4.7 to 5.0. The result of directional selection revealed that the *prsc* numbers in melon fly were greater than those of wild populations (Fig. 5). Various phenotypes and their distribution patterns offered a chance for genetic

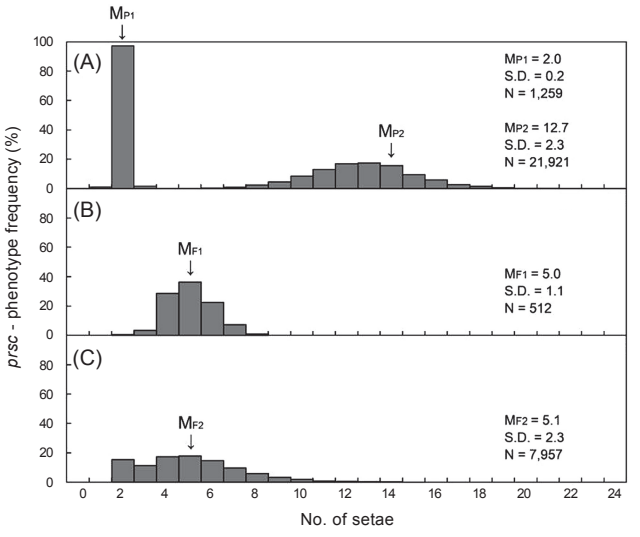


Fig. 4. Histogram of phenotypes frequencies with different numbers of *prsc* from (A) the parental strains, (B) the F_1 and (C) the F_2 generations in the 2-*prsc* × 14-*prsc* phenotypes cross test. *prsc*: prescutellar bristles.

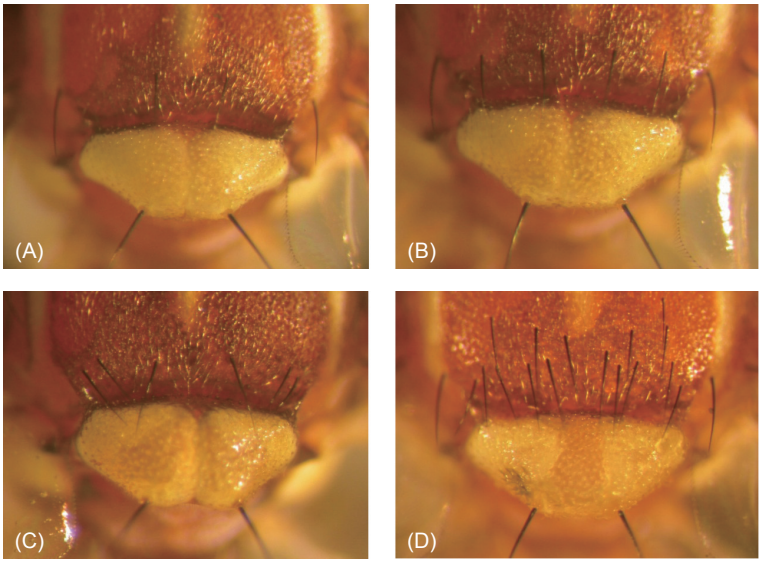


Fig. 5. Melon flies with various numbers of *prsc* by selection. (A) The 2 *prsc* phenotype or the wild type; (B) The 4 *prsc* phenotype; (C) The 6 *prsc* phenotype; and (D) The 16 *prsc* phenotype.

investigation on possible control mechanism.

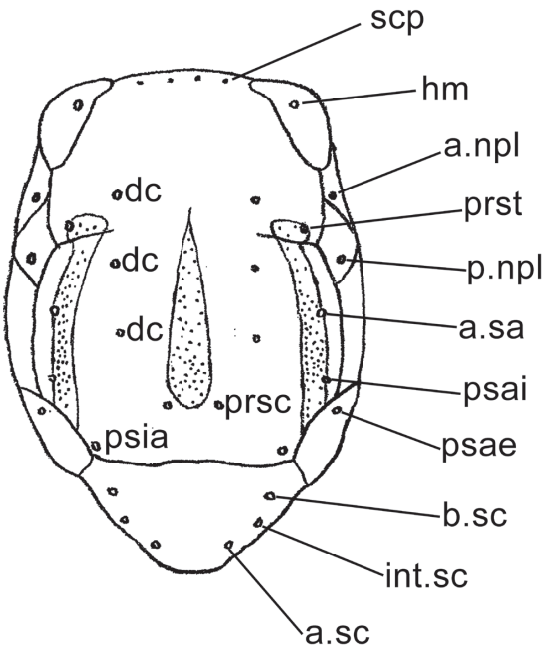
The quantitative genetics of *prsc* became in sight because a tendency of normal distribution of *prsc* phenotypes is evident: the more or the less *prsc* number phenotypes from the center or major phenotype were always gradually lowered in frequency count and formed a bell shape distribution. Hence, the mean, the standard deviation, and range of *prsc* were suitable to characterize the strain as well as for the genetic analysis.

The genetic analysis of mean, variance and the range of *prsc* in F_1 and F_2 of both 2-*prsc* × 12-*prsc* and 2-*prsc* × 14-*prsc* crosses fit the criteria of corn ear length study of Emerson & East (1913); hence, confirming that the quantitative genetic mechanism is responsible for the *prsc* numbers in melon fly. Why the 2-*prsc* phenotype dominates the wild population at a percentage more than 95% and whether this fact implies that the contribution of the polygenes in the continuous trait may not be equal in their dominance are interesting. This possibility is evident in the genetic studies, as the *prsc*-phenotype distributions of F_2 were slightly deviated from the normal distribution, in favor of 2-*prsc* phenotype or the dominated wild type. The environmental influence, an important consideration in quantitative genetics, is minimum due to all melon flies were reared in the same laboratory condition with uniform diets, temperature, humidity, photoperiod, etc..

CONCLUSIONS

This investigation is tried to find out the genetic control mechanism of *prsc* number in the thorax of melon fly. Through the directional selection of strains with different phenotypes and the genetic tests by crossing different phenotypes for F_1 through F_2, it is possible to conclude that *prsc* is controlled by quantitative genetics based on following results: (1) In F_1 progeny, the mean of *prsc* is intermediate between two parental phenotypes. (2) The mean of *prsc* in the F_2 progeny is statistically equal to the mean of F_1. (3) The standard deviation of F_2 showed more variability around the mean of *prsc* than the F_1 did. (4) The extreme values for the *prsc* in the F_2 progeny extended to the two parental values than did the extreme values of the F_1.

APPENDIX



Appendix The schematic setae of mesonotum for the melon fly.

scp: scapular bristles; *hm*: humeral bristle; *a.npl*: anterior notopleural;
prst: praestutural bristles; *p.npl*: posterior notopleural bristles;
a.sa: anterior supra-alar bristles; *psai*: posterior supra-alar bristles;
psae: posterior external supra-alar bristles; *prsc*: prescutellar bristles;
psia: posterior intra supra-alar bristles; and *b.sc*: base scutellar bristles.

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瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 中胸背板剛毛之數量遺傳機制

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摘要

鄭允、王志賢、黃毓斌、江明耀。2014。瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 中胸背板剛毛之數量遺傳機制。台灣農業研究 63(3):179–187。

野生瓜實蠅 [*Bactrocera cucurbitae* (Coquillett)] 中胸背板之剛毛數 (prescutellar bristles; *prsc*) 有 95% 個體為 2，此外尚有少數個體為 0–4 隻剛毛，合計約 5%。為瞭解此多型態 (polymorphism) 之控制機制，以室內汰選方式進行 2-*prsc* 及 4-*prsc* 單一形態型品系之純化，經過一百代以上，仍無法選出，反而在 4-*prsc* 汰選中觀察到有更多剛毛個體的出現。而 2-*prsc* 之汰選結果平均數為 2.0 ± 0.7，範圍為 1–3；4-*prsc* 之結果平均數為 4.0 ± 0.4，範圍為 1–7，因此再汰選多剛毛品系，所得結果為平均數為 12.7 ± 2.3，範圍為 8–19。所有汰選均得到由一系列連續分布的多形態個體所組成之族群，此等多形態型組成有可能為數量遺傳 (quantitative genetics) 機制所造成。因此，進行遺傳測試求證，以 2-*prsc* 為父本，分別與 12-*prsc* 及 14-*prsc* 之母本雜交出 F_1 子代後再自交成 F_2 子代，計算其 *prsc* 不同數目型之個體數，求取平均值、標準誤差及分布範圍。F_1 與 F_2 平均值以 *t*-test 進行差異顯著度測試，結果發現下列四項數量遺傳之佐證：(1) 兩項測試之 F_1 平均剛毛數分別為 4.7 及 5.0，均介於原父本及母本之間；(2) 兩項測試之 F_2 平均剛毛數分別為 4.7 及 5.1，在 *t*-test 顯示與 F_1 之平均值相同無顯著差異；(3) F_2 子代剛毛數變異係數 (Coefficient of variation) 均較 F_1 子代者為大，分別為 40.1% > 19.5% 及 45.9% > 21.7%；(4) F_2 子代剛毛數分佈之兩端極端可達父本及母本之範圍，分別為 2–15 *prsc* 及 1–17 *prsc*。本研究與 1913 年 Emerson 及 East 之玉米穗長度數量遺傳之經典測試結果均能吻合。因此，推論瓜實蠅中胸背板剛毛數之控制機制為數量遺傳，也與另一雙翅目之 *Drosophila* 屬剛毛數控制機制為數量遺傳相同。

關鍵詞：瓜實蠅、中胸背板剛毛、數量遺傳分析、數量策略。

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