THE PUPARIUM OF *RHAGOLETIS BASIOLA* (O.S.): VARIATION IN LENGTH AND FORM

(TRYPETIDAE, DIPTERA)

W. V. BALDUF
Urbana, Illinois

INTRODUCTION

While observing insects associated with hips of roses in 1945–1951, I reared numerous puparia of the rose hip fly, *Rhagoletis basiola*. Seeing that the puparia varied decidedly in length and form, I measured several thousands in order to determine the nature, and possibly the causes, of the observed variation. The materials measured were acquired in a period of six years from the states of Ohio, Illinois, Wisconsin, and Minnesota where they originated in five species of *Rosa* listed in table 2.

Two kinds of mensurational data are presented for interpretation in the pages below. One pertains to length only of the puparia, the other to the length and maximum diameter of the insect. Since the oval straw-colored bodies are normally perceptibly depressed, the diametric measurement refers to the width or transverse dimension. The data concerned with length only impart some idea of size, whereas those pertaining to length and diameter combined have to do with form or volume.

METHOD

Table 1 illustrates the method employed for analyzing the mensurational data. In the upper half a sample of puparia is classified according to variation in length, and in the lower part another sample is divided on the basis of volume, shape or form. Both "length" and "form" data are distributed into eight parallel groups of equal extent or inclusiveness. The length groups are delimited in terms of millimeters: *e.g.*, group 1 consists of puparia whose length ranges from 3.0 to 3.49 mm., and group 8, of puparia 6.5 to 6.99 mm. long, while the limits of groups 2 to 6 intergrade between these extreme groups 1 and 8. By contrast, the bounds of the eight form groups are stated in terms of relative lengths and diameters, *i.e.*, ratios. For example, the puparia of form group 1 have extreme ratios of 1.77:1 and 1.99:1. These are the most robust, but not necessarily the smallest puparia. Here 1.99 signifies the puparia are 1.99 times longer than their maximum breadth, and not 1.99 mm. long by 1.0 mm. wide. The distribution of the puparia composing any sample among the two series of groups is given in terms of percents. As illustrations, note that 64.32 percent of the puparia parasitized by *Halticoptera* (table 1) fall into length group 3, whose limits are 4.0 and 4.9 mm., and 37.21 percent of a comparable sample occur in form group 3, whose extreme ratios are 2.25:1 and 2.49:1.

RESULTS

Samples analyzed.—Altogether 15 samples of puparia, totalling approximately 6,800, were analyzed to determine their percentile distribution with reference to length and form, according to the above method. Three of these are presented in full in table 1. The "majorities" of all the 15 samples will be found in table 2.

General nature of variation in length.—When analyzed with reference to their percentile distribution among the eight length groups, the 15 samples are found to constitute three distinct, although unequal, classes. In characterizing these classes, I consider only the numerical "majorities" of the several samples instead of the total puparia: this for the reason (1) that the majorities are the most signi-

TABLE 1

Puparia in relation to parasitism

Effect of parasites on length of 1149 puparia

Parasites in the puparia	Puparia measured	Numerical limits of eight groups based on length of puparia, in mm.							
		puparia 3.0–3.49 mm. long	puparia 3.5–3.99 mm. long	puparia 4.0–4.49 mm. long	puparia 4.5–4.99 mm. long	puparia 5.0–5.49 mm. long	puparia 5.50–5.99 mm. long	puparia 6.0–6.49 mm. long	puparia 6.5–6.99 mm. long
Halticoptera	243	5.95%	21.08%	64.32%	8.65%	0.00%	0.00%	0.00%	0.00%
Opius	373	1.17	8.19	44.73	38.30	7.31	0.30	0.00	0.00
None	533	2.06	7.32	18.76	30.20	30.02	11.26	0.38	0.00

Effect of parasites on form of 1059 puparia

Parasites in the puparia	Puparia measured	Numerical limits of eight groups based on form, i.e. ratio of length to diameter, of puparia							
		1.77: 1 to 1.99: 1	2.0: 1 to 2.24: 1	2.25: 1 to 2.49: 1	2.50: 1 to 2.74: 1	2.75: 1 to 2.99: 1	3.0: 1 to 3.24: 1	3.25: 1 to 3.49: 1	3.50: 1 to 3.75: 1
Halticoptera	230	0.00%	26.74%	37.21%	23.83%	4.65%	6.41%	1.16%	0.00%
Opius	319	1.36	36.27	49.14	11.87	1.36	0.00	0.00	0.00
None	510	2.94	25.89	45.49	19.60	4.51	1.18	0.00	0.39

ficant fractions as indicators of the nature and amount of variation among the puparia, and (2) their variational values are more conveniently described than those of the entire samples. With few exceptions, the minorities of the samples prove to fall into all the length groups that do not contain the majorities, but they vary with reference to the number of percents per group.

Class I. The majorities of the 12 samples (table 2) which constitute this class, range from 72.65 to 85.99 percent, with a spread of only 13.34. Moreover, all these samples fall into the same length groups 3, 4 and 5, whose inclusive extremes are 4.0 and 5.49 mm. Therefore, while not strictly uniform as to size of their component puparia, the samples are fairly compact or homogeneous as components of their class. The antecedent larvae, from which the puparia developed, origi-

TABLE 2

Percental Values of "Majorities," in Groups 2–6, based on Length of Puparia

Distribution of the majorities of the 15 samples as based on the length of the puparia

	Limits of "majority" groups 2–6, in mm.				
	No. 2	No. 3	No. 4	No. 5	No. 6
Sources of samples	3.5–3.99	4.0–4.49	4.5–4.99	5.0–5.49	5.5–5.99
			Class I		
Northeastern Minnesota		80.67 per cent, groups 3–5			
Northwestern Wisconsin		78.45 per cent, groups 3–5			
East Central Illinois		75.64 per cent, groups 3–5			
Random areas		78.92 per cent, groups 3–5			
Year 1945		85.99 per cent, groups 3–5			
Year 1946		81.49 per cent, groups 3–5			
Year 1949		72.65 per cent, groups 3–5			
Year 1950		83.06 per cent, groups 3–5			
Rosa acicularis		83.34 per cent, groups 3–5			
Rosa blanda		76.09 per cent, groups 3–5			
R. blanda and *carolina*		77.08 per cent, groups 3–5			
Parasite-free puparia		78.98 per cent, groups 3–5			
			Class II		
Rosa palustris, Urbana			83.10 per cent, groups 4–6		
			Class III		
Parasitized by *Halticoptera* sp.	85.40 per cent, groups 2–3				
Parasitized by *Opius* spp.		83.03 per cent, groups 3–4			

nated as follows (table 2): (a) in three distinct geographical locations; (b) in four separate years; (c) in three species of roses; and (d) in miscellaneous localities, years and roses. The latter (d) has reference to sample 12: all the puparia were dissected after measurements and thereby shown to be free of parasites. Puparia from a, b, and c contained parasites in varying numbers.

About 93 percent of the total lot of more than 6800 puparia concerned in this investigation are included in the 12 samples of Class I. That so great a preponderance of the over-all lot of individuals is so nearly similar in length, despite the diversity of places, times, rose hosts and rates of parasitism, signifies that the sum of the environmental influences was comparatively constant in most times and natural conditions.

Class II (table 2). This fraction of the over-all lot of puparia consists of a single sample. It was derived from the hips of *Rosa palustris* cultivated under somewhat ideal conditions at Urbana, Illinois. This class differs from Class I

in that its majority of 83.10 percent belongs to length groups 4, 5 and 6; *i.e.*, its puparia measure from 4.5 to 5.99 mm. in length. An additional 12.09 percent of this sample fall into group 7, measuring up to 6.5 mm. Accordingly, these puparia average decidedly larger than those of Class I, and are the largest I have obtained from any and all sources in the years 1945–1951.

Class III (table 2). This class consists of two samples of puparia. Both were shown, by means of dissection, to be wholly parasitized, one by the pteromalid chalcidoid, *Halticoptera rosae* Burks, the other by the braconids, *Opius balduſi* Mues. and *O. rosicola* Mues. The "majority" of the puparia inhabited by *Halticoptera* amounts to 85.40 percent, and falls into length groups 2 and 3. Being only 3.5 to 4.45 mm. long, this majority contains the smallest puparia known to me. The majority of the puparia attacked by *Opius* spp. adds up to 83.03 percent and belongs to length groups 3 and 4, which measure 4.0 to 4.99 mm., hence are slightly larger than those parasitized by *Halticoptera*.

Variation among the majorities of the samples.—In addition to the variations described for the three classes above, diversity in length of the puparia is shown also in the percentile distribution within the majorities of the 15 samples. In some instances, these groupwise differentials vary to a significant extent. Such variation is exemplified by the following cases.

In the sample (table 2) obtained at Eaglesnest, near Ely, in north-eastern Minnesota, the percentile distribution among length groups 3, 4 and 5 was 30.78, 28.82 and 21.07, respectively (total 80.67 percent). This tendency of the puparia to the small groups 3 and 4 may signify a fairly high rate of endoparasitism, or other factor strongly reducing size of this fly stage. In the sample from northwestern Wisconsin, but mostly Chetek, the groupwise percentile distribution was 22.62, 25.44 and 30.39 (total 78.45), meaning that this particular sample averaged larger than those from Eaglesnest, and possibly indicates a lower rate of parasitism, or other restricting factor. In the sample from eastern Illinois, but largely Champaign County, the pattern was 12.18, 29.17 and 34.29 percents (total 75.64). Accordingly, the puparia from Eaglesnest averaged smaller than those from Wisconsin, and both these proved smaller than the sample that originated in Illinois.

Similar differences of a more or less distinct degree appear in all the 15 samples. I do not interpret these diversities in percentile patterns in the majorities to be fortuitous, but believe they represent the varied effects of several factors whose individual influences on the growth of *Rhag. basiola* differ in magnitude with time and circumstance.

Comparison of variation in (a) length and (b) form.—Considering the 15 samples as a whole, the puparia show an extreme variation in length of approximately 3.00 to 6.90 mm., with the "majorities" distributed over the five length groups numbered 2 to 6 (table 2). But when treated for variation in form, expressed in terms of ratio of length to diameter, these majorities of the samples fall into three adjacent form groups, numbered 2, 3 and 4 (table 1), whose extreme ratios are 2.00:1.00 and 2.74:1.00. This relative uniformity in the form samples is reflected also by the ratios presented in table 3, where the data given under "average form" are found to be closely similar despite (a) the absolute differences in the samples as regards parasitism, and (b) the origin of the three lots, A, B and C, in various habitats.

What is the meaning of the observed differences between length and form of the puparia? On first thought, it may seem that length and form are distinct aspects of size, which might be interpreted to signify either the operation of distinct causative factors for length and form, or that one and the same factor or factors may effect a proportionately greater change in the diameter than in the length of a puparium. However, the hypothetical two "types" of variation are basically identical, and only appear to be distinct due to the two distinct mathematical devices employed to break the samples down into the length groups and

form groups, respectively. Ratios are calculated by dividing length by diameter, and they therefore represent values intermediate between the two basic dimensions. The extremes of length and width are minimized in this method of expressing variation, and, as a consequence, the "majorities" of the several samples, when classified on the basis of form, appear decidedly more homogeneous with one another than when divided according to variation in the longitudinal dimension only.

As a means of expressing variation, length is preferred over form, or ratio, for the reason that it serves to emphasize rather than minimize the lines of demarcation existing in the puparia. The diverse differences revealed by measurements of length serve further to indicate, or even to identify, the ecological factors that operate as causes of variation, whereas the data on form or ratio give comparatively little intimation of such causes.

Causes of variation in length.—Table 2 suggests clues as to the identity of factors that may be associated, in causative relations, with variation in length of the puparia. However, the observation that 12 of the samples (Class I) belong to groups 3, 4 and 5, with extreme lengths of 4.0 to 5.49 mm. and are therefore comparatively uniform in length, indicates that the geographical sources, years of acquisition, the plant hosts, *Rosa acicularis*, *blanda* and *carolina*, and the parasite-free condition of the twelfth sample, did not, as entities, exert major influences on the length of the puparia, or it indicates that some of the factors involved in each situation operated counterwise to others to produce the observed uniformity. By contrast, Classes II and III stand distinctly to the right and left, respectively, of Class I (table 2). Hence, it appears that the greater length of the puparia of Class II may be attributed to the favorable qualities of their host, *Rosa palustris*, and that the smaller lengths of those in Class III correlate with parasitism by *Halticoptera* and *Opius*.

Rosa palustris as a cause of large size.—That puparia of Class II exceed in size those of Class I is due largely to the influence of *R. palustris* appears certain when we examine the circumstances under which this particular rose grew. It was distinctive in that it was cultivated in rich soil, fully exposed to the summer sun, mature in size, vigorous of stem and lush in foliage. The hips grew large, and when ripe in September-October, had become a rich brownish-red color, and probably contained a high percent of vitamin C. Melville and Pyke (Proc. Linn. Soc. Lond., p. 159, 1946) have shown that this vitamin attains its maximum volume when the hips of the several British species are ripe late in the growing season. I have found that the larvae of *Rhag. basiola* are, generally, approaching their maturity at that time of year. It may therefore be supposed that the vitamin indirectly contributes significantly to the exceptionally large size of the puparia derived from this clump of roses.

However, it is improbable that the flourishing state of these plants is the sole cause of the large puparia. Dissections of sample puparia from this source revealed that *Halticoptera* did not occur and *Opius* spp., present in small numbers elsewhere in eastern Illinois, had not invaded the garden where *palustris* thrived. That parasitism effects a reduction in the length of puparia is apparent from the position of the parasitized sample in Class II (table 2).

Parasites as factors in variation (table 1).—Three samples, totalling 1149 puparia, obtained largely from northwestern Wisconsin and northeastern Minnesota, were dissected to determine the species of parasite affecting them, and analyzed with reference to the bearing of these internal enemies on variation in length of the puparial host. One sample consists of puparia which proved to contain the first instars of *Halticoptera*; a second contained various instars of *Opius* spp.; and the third was free of all parasites. Data in table 2 show that the majorities of the puparia parasitized by *Halticoptera* fall into groups 2 and 3. Being only 3.5 to 4.49 mm. long, these were the smallest puparia discovered, which

indicates that this parasite effects an extreme reduction in the size of the host. That *Opius* spp. did not reduce the host to the same extent as *Halticoptera* is shown by the position of the majority puparia in length groups 3 and 4, which measured 4.0 to 4.99 mm. How this differential between *Opius* and *Halticoptera* is achieved may be seen below from their distinctive impacts on the host.

The two genera are similar in their host relations in that both persist as first instars while the host larvae feed and grow to maturity. Both feed sparingly in the larvae of *Rhagoletis* and thereby enable the host to mature and to pupate. However, the similarity ends here. The female *Opius* places the zygotes in the larva of the fly, usually the second and third instars, whereas *Halticoptera* oviposits into the embryo of the host fly, where the first instar parasite hatches. Accordingly, the first larva of *Halticoptera* exerts its retarding effect on the development of both the embryo and all the three instars of the host, whereas the first larva of *Opius* works counter to only the second and third instars, and sometimes

TABLE 3

Parasitism in relation to form based on a sample of 1264 puparia

Lots and sources of puparia	Parasitized by *Halticoptera*		Parasitized by *Opius*		Not parasitized	
	Extremes of form	Average form	Extremes of form	Average form	Extremes of form	Average form
Lot A, *Rosa acicularis*, Eaglesnest, Minnesota	3.0 x 1.5 and 4.8 x 2.0 mm.	249 puparia 4.02 x 1.76 mm., or 2.28: 1	3.05 x 1.15 and 5.7 x 1.95 mm.	340 puparia 4.34 x 1.9 mm., or 2.28: 1	3.0 x 1.3 and 6.0 x 2.5 mm.	131 puparia 4.93 x 2.12 mm., or 2.33: 1
Lot B, *Rosa blanda*, Eaglesnest, Minnesota	3.1 x 1.1 and 5.0 x 2.0 mm.	98 puparia 4.12 x 1.74 mm., or 2.31: 1	3.3 x 1.3 and 5.1 x 2.2 mm.	25 puparia 4.25 x 1.76 mm. or 2.42: 1	3.8 x 1.2 and 5.9 x 2.8 mm.	107 puparia 4.64 x 2.0 mm. or 2.32: 1
Lot C, *R. blanda* and *carolina*, Chetek, Wis.	3.05 x 1.05 and 4.9 x 2.0 mm.	187 puparia 3.94 x 1.65 mm., or 2.39: 1	3.4 x 1.2 and 5.1 x 1.92 mm.	18 puparia 4.2 x 1.74 mm., or 2.41: 1	3.01 x 1.1 and 5.65 x 2.5 mm.	109 puparia 4.53 x 1.92 mm., or 2.36: 1

probably only the third. This differential effect on the growth of the host, as reflected in the length of the puparia, comes about despite the somewhat larger size of the first instar *Opius*. It therefore appears that the relatively subdued but long-time attack of the smaller *Halticoptera* on embryo and larva of *Rhagoletis* more than offsets the advantage held by the larger and more aggressively adapted first instar of *Opius*.

That parasites cause reduction in length of the puparia may be inferred also from the above observation that the exceptionally large puparia from *Rosa palustris* contained no *Opius* or *Halticoptera*. The data presented in table 3 pertain to the forms of puparia parasitized by these same enemies and of puparia found to be free of the same. The "not parasitized" samples of all the three lots, A, B, and C exhibit greater ranges in form than do those affected by the endoparasites, showing again that endoparasites have a retarding effect on the size of the puparia.

Other factors related to variation in length.—While rose hosts and parasites appeared to be the dominant causes of variation in these samples of puparia of *Rhag. basiola*, they are quite certainly not always the sole, nor even the major factors.

As stated above, the absence of parasites in the instance of *Rosa palustris* at Urbana clearly favored the record large size of the puparia, and it is very likely that other adverse influences supplemented the parasites in the production of the inordinately small puparia in Minnesota and Wisconsin. Evidence of three such supporting factors came to my notice in the latter areas between 1945 and 1951.

One significant influence observed repeatedly at Eaglesnest, Minnesota, concerned the inherent bony hardness of the unripe hips of *Rosa blanda*. This texture of the hypanthium frequently thwarted *Rhag. basiola* in her efforts to prepare pits for the reception of eggs, and also often rendered the establishment and feeding of the first instars difficult, with the result that some surviving individuals did not attain normal size and consequently became puparia of meager length.

Again, eggs of the rose hip fly may be inserted into over-ripe hips, particularly in late summer. As a consequence, the larvae may be obliged to complete their advanced instars by feeding on deteriorating hypanthium, and are therefore undernourished and develop to undersized puparia. Some of the smallest puparia I have seen occurred under such conditions in hip jars in October. Dissection showed that some such small individuals contained no parasites, hence their reduced size was probably often attributable to their meager food.

Thirdly, it is very probable that the advent of drought conditions, which prevailed late in some summers, and particularly in the area of Chetek, Wisconsin, caused high percents of the hips of *R. blanda* and *carolina* to shrivel and toughen. This texture of the hypanthium rendered feeding by the larvae difficult, with the result that individuals, which barely attained maturity, eventuated as undersized puparia. While *Halticoptera* and *Opius* occurred here, they did not affect all the small puparia. This fact indicates that drying hips alone are significant factors in determining size of the insects inhabiting them.

Complexes of factors.—From the above consideration of environmental factors, it becomes apparent that climatic and biotic influences contribute to variation in the length of the puparia of *Rhag. basiola*. Moreover, it is clear that the factors do not operate only as individuals but largely as complexes. Among these associated influences generally occur both positive and negative types which, by their inherent contradictory nature, work counter to one another. These conflicts tend to prevent any one factor from exerting its full influence, either to produce puparia of exceptionally large, or small, size. This fact is indicated in Class I, defined above, whose "majorities" embraced puparia of average lengths, *i.e.*, 4.0 to 5.49 mm., and, moreover, contained 93 percent of all the puparia involved in this study.

The data pertaining to this investigation indicate further that the several components, such as rose hosts, parasites and drought, obviously vary in the magnitude of their influences from place to place and from year to year. Under some kinds of environmental conditions, certain factors, for example *Rosa palustris* and the parasites, *Halticoptera* and *Opius*, exert major controls over length. However, in other times and places, rose hosts and parasites perform at various reduced levels of effectiveness.

This principle of fluctuating influence was illustrated in northeastern Minnesota in the decade of 1943–1953. Dissections of larvae and puparia of *Rhag. basiola* revealed that both *Halticoptera* and *Opius* sank to low levels of performance in some of the years, while in others, one or other of them attacked almost 100 percent of the host samples examined.

SUMMARY

The puparia of *Rhagoletis basiola* are subject to wide variation in length and form. Length varied from 3.0 to 6.9 mm., form from a ratio of 1.77:1.00 to 3.75:1.00. Length and form are merely two ways of stating the amount of variation. Form, *i. e.*, ratio of length to diameter, has the effect of minimizing length values, and therefore is less desirable as a method of expressing variation. Ac-

cordingly, form also has less value as an indicator or identifier of the causal factors of variation.

Factors identified as causes in this study are ecological, and both climatic and biotic. Drought may indirectly influence the size of puparia through its adverse effects on the hips and the contained growing larvae. Biotic factors instrumental as determinants of size include (1) bony texture and (2) deteriorated condition of the hypanthium inhabited by the larvae, (3) the size of the hip, or quantity of food available to the larvae, (4) its content of vitamin C, and (5) the hymenopterous parasites, *Halticoptera* and *Opius*. Of these factors, the parasites exert the most decisive effect on the size of the puparia.