FINAL REPORT **DURATION**: 1 year

Project Title: DNA and morphometric diagnosis for apple and snowberry flies

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Organization: USDA-ARS	**Contract Administrator:** Bobbie Bobango		
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Item	**Year 1: 2007**	**Year 2:**	**Year 3:**
Salaries			
Benefits			
Wages	12,700		
Benefits	1,270		
Equipment			
Supplies	800		
Travel	230		
Miscellaneous			
Total	**$15,000**		

Budget 2:

Organization: University of Notre Dame	**Contract Administrator:** Rick Hilliard		
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Item	**Year 1: 2007**	**Year 2:**	**Year 3:**
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies	9,000		
Travel	1,000		
Miscellaneous			
Total	**$10,000**		

Objectives:
Our objectives are to discover diagnostic morphological and molecular differences between apple and snowberry flies and to measure genetic differences among Washington populations of the apple maggot fly to detect possible hawthorn and apple host races and to support quarantine and remediation programs in Washington.

1. Re-evaluate morphometric variation between apple and snowberry maggot
2. Seek diagnostic differences between apple and snowberry maggots using ISSR and RAPDS
3. Determine if host races of apple maggot exist in Washington using microsatellite variation.

Proposed Schedule of Accomplishments:

Objective	2007
1. Morphometrics	Rear flies from apple and snowberry; shape measures determined
2. ISSRs/RAPDS	Primers screened on 10 flies of each species
3. Microsatellites	Initial genotyping of flies from WA and eastern U.S.

Significant Findings:

● Various body structures of apple and snowberry maggot flies, including the ovipositors, generally were larger in apple maggot flies, but there was overlap in all of measures.

● Body measures within apple maggot flies depended on the location or host origin.

● Wing shape analysis was a highly promising method to separate apple maggot from snowberry maggot flies; wings of the two have different shapes based on principal components and canonical variates analyses.

● Twenty four RAPD primers were surveyed on composite and individual insects and of these five show promise for diagnostic gene regions. Additional primers are being analyzed.

● Use of microsatellite genetic markers suggests significant differences exist between apple maggot flies reared from apple and hawthorns in Washington.

● 60 PCR primers developed to amplify microsatellite loci for eastern populations of apple maggot fly also worked for western flies from Washington State.

● Six of the eight loci (all except p71 and p18) displayed significant allele frequency differences among the apple, black hawthorn, and ornamental hawthorn populations ranging on the order of from 10 to 25%. These data are consistent with the existence of apple, black hawthorn, and ornamental hawthorn host races in Washington state.

Results and Discussion:

Results

1. Re-evaluate morphometric variation between apple and snowberry maggot flies.

Body Measure Analyses. Apple maggot and snowberry maggot flies reared from different hosts and from different areas in Washington differed in body measurements (Table 1). Apple maggot flies from apple from western Washington (Vancouver and Skamania) did not differ, except in wing length, and usually did not differ from apple maggot flies from ornamental hawthorn from Puyallup. Apple maggots from all sources, however, were in general larger based in body measurements than snowberry maggots. Snowberry maggots from central Washington generally had larger body measurements than snowberry maggots from western Washington (Table 1). Although apple maggots were larger than snowberry maggots, there was considerable overlap in body measurements, for example, in wing lengths (data not shown). There was also overlap in ovipositor lengths (Fig. 1). Principal component analyses (PCA) using the 9 body measurements resulted in significant species effects: comparisons of means of principal component 1 show that apple maggots differed from snowberry maggots. Apple maggots from apple from western Washington (Vancouver and Skamania), from apple from Puyallup, and from ornamental hawthorn from Puyallup did not differ, and differed from apple maggots from ornamental hawthorn and from black hawthorn in western Washington. Snowberry maggots from central and western Washington also differed in means of principal component 1.

Wing Shape Analyses. Inspection of representative wings (Fig. 2) suggests the apple maggot wing is narrower and less rounded apically than the snowberry maggot wing. PCA using the 14 landmark data resulted in significant species effects for principal component 2 * principal component 1 (Fig. 3), showing that the wings of apple and snowberry maggots differ significantly in shape. Canonical variates analysis (CVA) for variate 2 * variate 1 using the landmark data (Fig. 4) resulted in even clearer separation of differences in the wing shapes of snowberry and apple maggot flies.

2. Seek diagnostic differences between apple and snowberry maggots using ISSR and RAPDS.
We screened 24 RAPD primers with Qiagen extracted DNA from Snowberry (SB) and Western Apple Maggot (WAM). To screen the primers a male and female from Snowberry and Western Apple Maggot were selected for PCR. If there was a difference in banding pattern then that primer was used again for three males and three females from SB and WAM. Of the 24 RAPD primers we found 6 to be too light or negative, 13 with the same banding pattern across species, and 5 that look promising by showing unique bands in one or more individuals of one of the two species. We are currently continuing to evaluate those five primers with additional individuals of each species to clarify the results. No additional funding will be required for the evaluation of the RAPD variation; however, during 2008 we will examine additional RAPD primers and several ISSR primers. Apparently diagnostic bands will be TA cloned, sequenced, and primers designed and tested.

3. Determine if host races of apple maggot exist in Washington using microsatellite variation.
One goal of the study was to score apple maggot flies collected from apple and hawthorn in Washington for microsatellite genetic markers to assess where host races of the fly exist in the state. The one year of funding allowed us to determine that all of the 60 pairs of PCR primers developed to amplify variable microsatellite loci for eastern populations of apple maggot, also worked for western flies from Washington State. A subset of eight loci were scored for approximately 25 flies each collected from apple, black hawthorn, and an ornamental hawthorn host in Washington (These eight loci are designated p71, p17, p11, p80, p39, p25, p18, and p13). Six of the eight loci (all except p71 and p18) displayed significant allele frequency differences among the apple, black hawthorn, and ornamental hawthorn populations ranging on the order of from 10 to 25%.

Discussion

1. Re-evaluate morphometric variation between apple and snowberry maggot.
Body Measure Analyses. Results in this study indicate that apple maggots are larger than snowberry maggots in Washington in all body measures and that there may be differences even within the species depending on the host and region in which fruit are collected. Size measures alone can separate the species. Some characters such as the head width and ovipositor lengths seem the least variable of the structures measured, but graphical analyses indicate that there is considerable overlap

in lengths of structures, including the ovipositor. Wasbauer (1963) and Wescott (1982) found that ovipositor lengths were longer in apple maggot than in snowberry maggot female flies. Wescott (1982) stated that there was a very small overlap in ovipositor lengths of flies from Oregon, with only five flies in the 'problem area' of 0.88-0.98 mm. Our data show that such overlap is not uncommon.

Wing Shape Analyses. Our results represent the first demonstration of differences in wing shape between apple and snowberry maggots. In particular, the apple maggot wing appears longer and narrower than that of the snowberry maggot. In cases where body measurements do not separate the species, wing shape may be used to separate them. An advantage of using wing shape over body measures may be that possible fruit quality effects on body size are reduced or eliminated; fruit quality unlikely affects wing shape. Free computer software from the State University of New York (SUNY) Stony Brook is available that can be used for wing shape analysis (website: http://life.bio.sunysb.edu/morph). In particular, the videodigitizing program TPSDig and the program CVAGen can be used for this purpose. CVA is a method that can be used to find axes along which fly groups are best discriminated. Scores for individuals can be used to assign unknown species to groups. Scores are then plotted to depict the distribution of specimens along axes. Use of such a technique may help solve the problem of difficult to identify flies that could affect quarantine decisions in commercial apple growing areas. Further work will be conducted to determine if wing shape analysis can identify flies that have body measurements that fall in overlap regions of frequency distributions. Also, the results using PCA and CVA need to be hold up to larger sample sizes. Inclusions of more samples in analyses are planned for the upcoming year.

2. Seek diagnostic differences between apple and snowberry maggots using ISSR and RAPDS.
Previous studies (Barcenas, Unruh, Yee) that examined sequence differences in a region of the mitochondrial gene CO-1 and in intron 1 of the nuclear gene, elongation factor alpha, show the potential to provide 95% or greater classification of snowberry maggot versus Western apple maggot. However, 95% is not diagnostic. A similar level of classification capacity has been recently reported by Jim Smith (Michigan State University), Mike Klaus (WSDA) and colleagues using amplified fragment length polymorphisms. We believe it is possible to discover a gene region or regions that show greater classification capacity and may be diagnostic in the absence of ongoing or a recent history of cross mating between these species. Further work will be conducted with RAPD and ISSR markers to discover such a marker. This will entail analyzing the five promising RAPD primers with larger numbers of flies collected from throughout the state and testing 6 ISSR primers. This work will be supported on base funding.

3. Determine if host races of apple maggot exist in Washington using microsatellite variation.
Microsatellite data are promising and consistent with the existence of apple, black hawthorn, and ornamental hawthorn host races of apple maggot in the state. However, further work is needed to increase the numbers of sample sites surveyed, the numbers of flies genetically scored, and the number of microsatellite loci analyzed at sites to confirm the findings suggesting apple and hawthorn host races in Washington state and to evaluate their source of origin. In particular, we now must concentrate on collecting and genetically scoring a series of sympatric sites from across Washington in which flies attacking apples, native, and ornamental hawthorns co-occur in close proximity. If these populations display the same pattern and degree of genetic differentiation displayed in the preliminary analysis, then the host race hypothesis will be verified. In addition, the success of the markers in potentially resolving frequency differences in what should be closely genetically related races of apple and hawthorn populations of apple maggot, provides a basis for expanding the analysis to snowberry maggot to determine whether the microsatellite loci can help distinguish these two taxa from each other.

Table 1. Mean measurements (± SE) (mm) of various structures of female apple maggot fly and snowberry maggot fly from different hosts and sites in Washington

	Host, AREA	Head Width	Inter-eye width	Wing Lngth	Wing Band 2	Wing Band 3	Hind Femur	Hind Tibia	Hind Tarsus	Ovipositor Length
AM (n = 40)	Apple, Vancouver WA	1.70 (0.01)a	0.66 (0.01)a	4.38 (0.04)b	0.82 (0.01)b	0.40 (0.01)a	1.45 (0.01)a	1.29 (0.01)a	1.33 (0.02)a	1.03 (0.02)a
AM (n = 58)	Apple, Puyallup	1.73 (0.004)a	0.64 (0.004)ab	4.50 (0.01)a	0.84 (0.006)ab	0.40 (0.002)a	1.45 (0.005)a	1.31 (0.005)a	1.36 (0.005)a	1.03 (0.004)a
AM (n = 50)	Ornmtl. Haw. Vancouver	1.52 (0.02)c	0.56 (0.008)d	3.93 (0.05)d	0.70 (0.01)d	0.35 (0.008)b	1.26 (0.02)c	1.10 (0.02)b	1.18 (0.02)b	0.99 (0.01)b
AM (n = 40)	Ornmtl. Haw. Puyallup	1.70 (0.01)a	0.63 (0.006)bc	4.44 (0.03)ab	0.86 (0.01)a	0.40 (0.008)a	1.42 (0.01)a	1.28 (0.01)a	1.35 (0.01)a	1.02 (0.01)ab
AM (n = 10)	Black Haw Vancouver	1.61 (0.03)b	0.60 (0.02)c	4.20 (0.08)c	0.76 (0.03)c	0.33 (0.02)b	1.32 (0.03)b	1.15 (0.03)b	1.21 (0.02)b	1.05 (0.02)a
SB (n = 66)	Snowberry C. WA	1.38 (0.01)d	0.51 (0.004)e	3.62 (0.03)e	0.74 (0.01)cd	0.36 (0.01)b	1.17 (0.01)d	1.04 (0.01)c	1.12 (0.01)c	0.79 (0.01)c
SB (n = 28)	Snowberry Vancouver	1.32 (0.02)e	0.50 (0.01)e	3.53 (0.05)e	0.68 (0.01)d	0.35 (0.01)b	1.12 (0.02)e	0.98 (0.01)c	1.08 (0.01)c	0.74 (0.01)d
One-way ANOVA[a] *F*		135.1	72.0	107.7	37.0	9.2	103.1	90.2	68.4	128.3
P		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

AM, apple maggot; SB, snowberry maggot;
[a]d.f. = 6, 285.
Means followed by same letters are not significantly different (LSD test, $P > 0.05$).

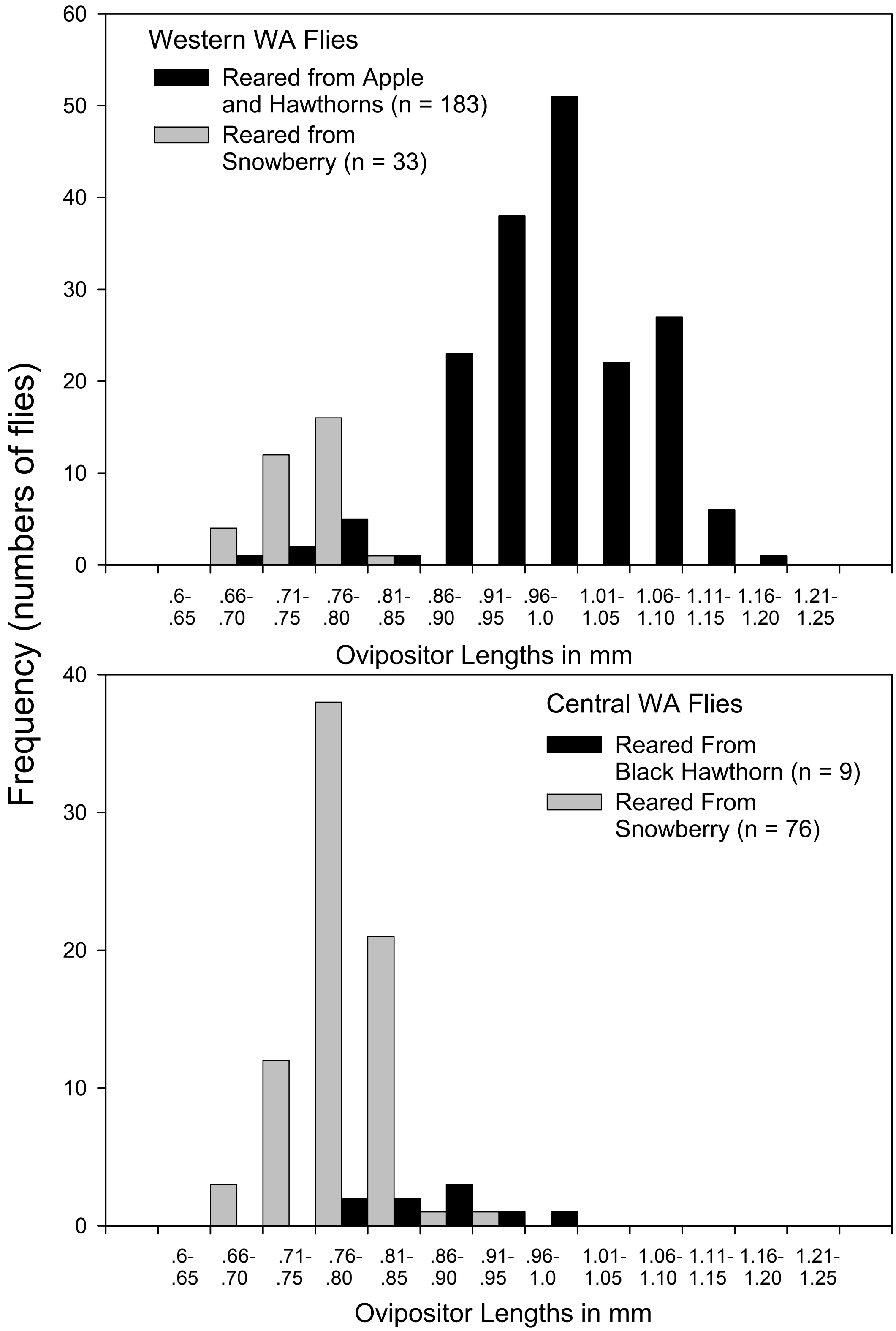

Fig. 1. Distributions of ovipositor lengths of snowberry and apple maggot flies.

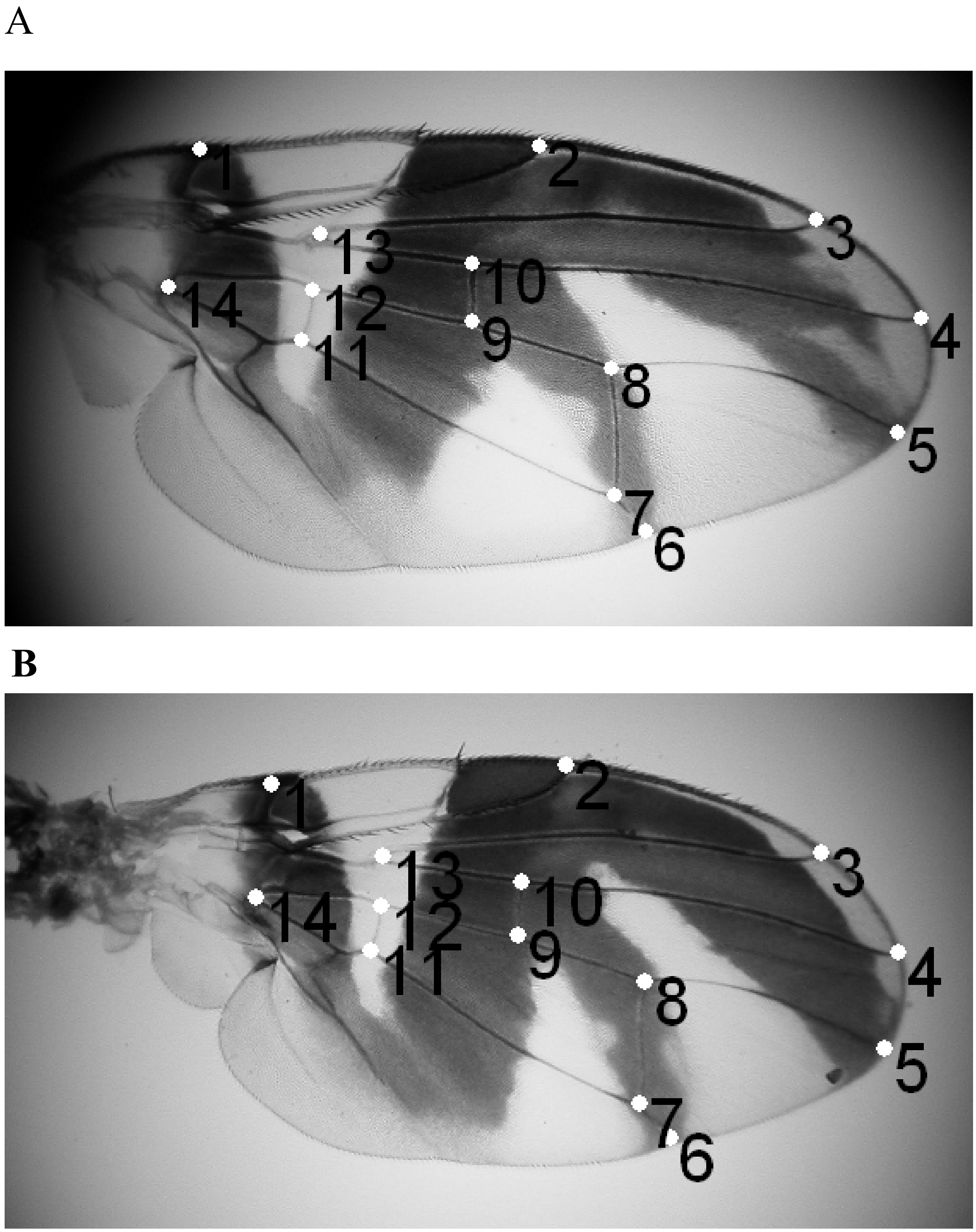

Fig. 2. Right wings of female (A) apple maggot (from western Washington) and (B) snowberry maggot (from central Washington), showing the 14 landmarks used for analyses.

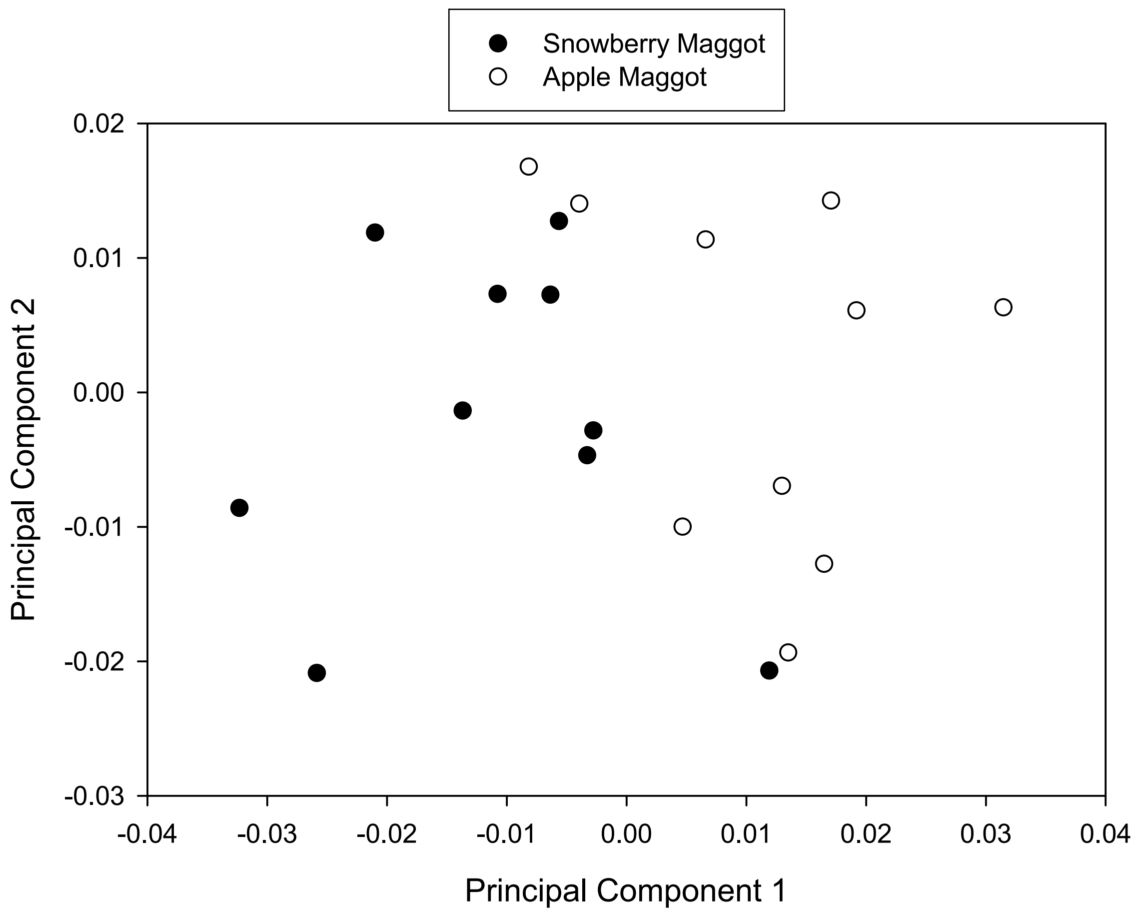

Fig. 3. Principal components analysis (PCA) of fly wing shapes: Plot of principal component 2 * principal component 1. Each X_1, X_2 value is from one fly.

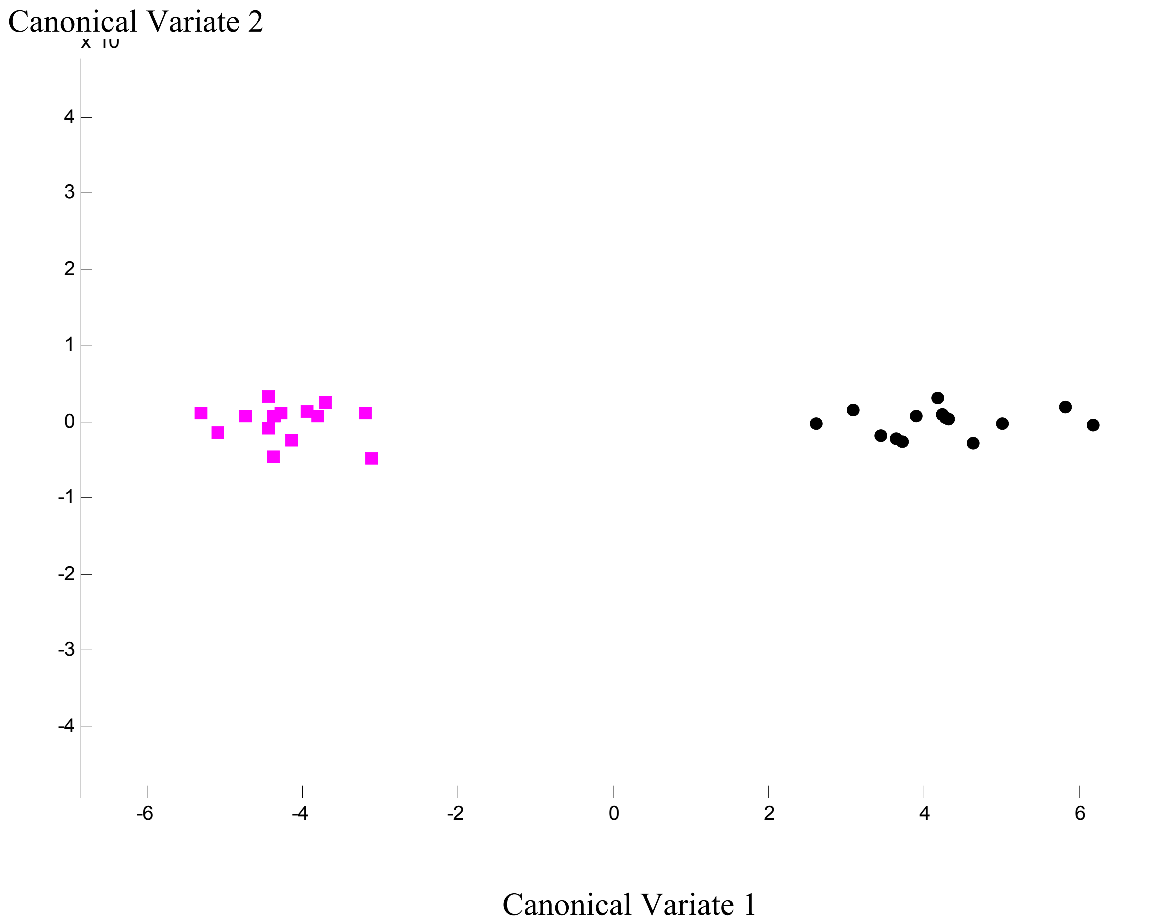

Fig. 4. Canonical variates analysis (CVA) of fly wing shapes: Plot of canonical variate 2 * canonical variate 1 of 30 fly wings. Each X_1, X_2 value is from one fly. Squares = apple maggot fly; circles = snowberry maggot fly.