AFLP analysis of genetic differentiation in *Cp*GV resistant and susceptible *Cydia pomonella* (L.) populations

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Abstract: The codling moth, *Cydia pomonella* (Lep., Tortricidae), is a significant pest of orchard crops such as apple and pear in Southern Germany, and can cause severe economic damage to apple crops. Due to resistance to conventional pesticides and the growing market for organic fruit, *Cydia pomonella* Granulovirus (*Cp*GV) has been used to control *C. pomonella* in Germany for over 10 years. Recently, populations exhibiting resistance to *Cp*GV have been reported. In this study, we have used amplified fragment length polymorphism (AFLP) markers to estimate genetic variations between eight different *C. pomonella* populations, which were obtained from different locations exhibiting varying levels of resistance to *Cp*GV. Three different AFLP primer combinations generated a total of 194 AFLP fragments, ranging from 57.84 to 424.11 bp, with an average of 59.23 amplified fragments per primer combination. The total number of segregating fragments ranged from 181 to 115 and resulted in a high loci polymorphism of 100% in most cases, except for two populations, where it was found to be 88.1% and 93.3%. An analysis of genetic variation based on the obtained AFLP markers resulted in high gene diversity (Hj) values, ranging between 0.2884 to 0.3508. Hj values also indicated a loss in gene diversity within a population over time. The Wright Fixation Index (F_{ST}) values indicated a low to moderate genetic differentiation in the populations. The cluster analysis (UPGMA), based on genetic distance values, showed that the majority of *C. pomonella* populations from different locations were clearly distributed into distinct groups and showed a large genetic variability.

Key words: *Cydia pomonella*, *Cydia pomonella* granulovirus, *Cp*GV, AFLP, genetic diversity

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The codling moth, *Cydia pomonella* (Linnaeus) is a major worldwide pest of apple, which also can attack pear and walnut. Historically, *C. pomonella* has developed resistance to conventional pesticides used for its control. Driven by the increasing occurrences of resistance of *C. pomonella* populations to conventional pesticides and the demand for organic products, *Cydia pomonella*-granulovirus (*Cp*GV) has increasingly been used in orchards as a control method. In 2002 a grower observed a lack of control of the codling moth population in spite of repeated applications of *Cp*GV. Individuals from this location in South Baden as well as two locations from around Lake Constance were sampled and bioassayed. The population of South Baden and Lake Constance II were observed to be significantly less sensitive to *Cp*GV, with LC_{50} values that were 1,000 times higher (South Baden) and 500 times (Lake Constance II) than that of the sensitive strain (Fritsch & al. 2005). This study uses AFLP markers to investigate the genetic differentiation in populations of *C. pomonella*, in an effort to gain a better understanding of the genetics behind the rise of this resistance.

Materials and Methods

DNA extraction, purification and all AFLP protocols were carried out as per the protocols developed by Reineke et. al. (1998). The primer combinations of Eco15 x Mse9, Eco16 x Mse21, and Eco17 x Mse20 were selected for AFLP analysis. The resulting gels were analyzed using GelCompar. From the fragment data, a binary matrix was composed using Dice Index and was further analyzed with AFLP-SURV 1.0 software, using the Lynch & Milligan method (Vekemans 2002). To account for the dominant nature of the

AFLP markers, allele frequencies were estimated using a Bayesian method (Holsinger & al. 2002) with a uniform prior distribution of allele frequencies. In addition, a cluster analysis was conducted based on the matrix complied by AFLP-SURV, and the final dendrogram was created using UPGMA with pairwise genetic distance data obtained from the three combined primer combinations using MEGA, version 3.0.

Table 1. Information on geographical location, resistance or susceptibility and year collected

Lab	Stage	Location	Location	Resistant or Susceptible	Year
BRU	Adults	BW-FN	Baden-Württemberg, Bodensee	**Resistant** Confirmed in laboratory. Bodensee II and Lake Constance II in publications. Heavy CpGV selection pressure.	2005 Earlier generation than 6BR.
6BR	Adults Larvae	BW-FN	Baden-Württemberg, Bodensee	**Resistant** same as above	2006
MB4R	Adults	BW-FI	Baden-Württemberg, South Baden, Fischingen / Rhine Valley	**Resistant** Confirmed in lab under heavy selection pressure from CpGV.	2005
LR	Adults	SL-SA	Saarland, Saarwellingen.	**Resistant** same as above	2004
NS	Larvae	BW-SN	Baden-Württemberg, Stuttgart North, Mühlhausen	**Susceptible** Wild population from untreated Streuobst. Under NO selection pressure from CpGV.	2005
ES	Larvae	BW-SO	Baden-Württemberg, Stuttgart East, Grabkapelle / Rotenberg	**Susceptible** Wild population from untreated Streuobst. Under NO selection pressure from CpGV.	2005
HS	Larvae	BW-SH	Baden-Württemberg, Stuttgart Hohenheim, Riedenberg and Heumaden S.	**Susceptible** Wild population from untreated Streuobst. Under NO selection pressure from CpGV.	2005
AL	Larvae	Lab	Hohenheim laboratory strain	**Highly susceptible** Under NO selection pressure from CpGV.	2005

Results and Discussion

A total of 67 *Cydia pomonella* individuals from 8 different populations and 3 primer combinations were included in the statistical analysis. Fragment size analyzed ranged from 57.84 bp to 424.11 bp. The level of polymorphism detected was quite high (Table 2). However, this is not unprecedented, as another study of populations in South Africa also reported high polymorphism (Timm & al. 2006). On the average, the Hj values (Table 2) indicate that the gene diversity in the susceptible wild populations is slightly higher than in resistant populations. An extremely interesting comparison occurs between the populations 6BR BWFN and BRU BWFN. which are from different generations of the same population. BRU BWFN is the older 'ancestor' population, and 6BR BWFN is the more recent generation. The Hj values indicate that the 'ancestor' population shows a higher gene diversity than the more recent population. It is a possibility that selection pressure from treatment with *Cp*GV could account for some of the rapid change and loss of gene diversity. The Fst (Table 3) values between 0.05 and 0.15 indicate a moderate level of genetic differentiation (Wright 1978) occurring in the analysis of all locations, all resistant and all susceptible. The level of differentiation was lower than that reported by Timm et. al. (2006), who attributed the high level in South African populations to the effects of genetic drift, as there were too few migrants between populations (Timm & al. 2006). In this study, while populations do show moderate to low levels of differentiation, this is probably more due to selection pressure than genetic drift. Unlike South Africa, in Germany there are many hosts and alternate hosts available. It is quite possible that these serve as 'stepping stones' and facilitate gene flow between populations. Gene flow between populations homogenizes the genetic composition and would account for the lower Fst values observed in this study. In the composite dendrogram (Figure 1), grouping by location sampled is quite strong. However, samples from a location did not always form a single cluster, but rather broke into smaller clusters or pairs. This is probably due to the high level of polymorphism and the high

level of gene diversity (Hj). The dendrogram further supports that a loss in gene diversity occurred between the two populations separated in time, as BRU BWFN exhibits the largest genetic distance between samples, whereas individual 6BR BWFN samples have a much smaller genetic distance between them.

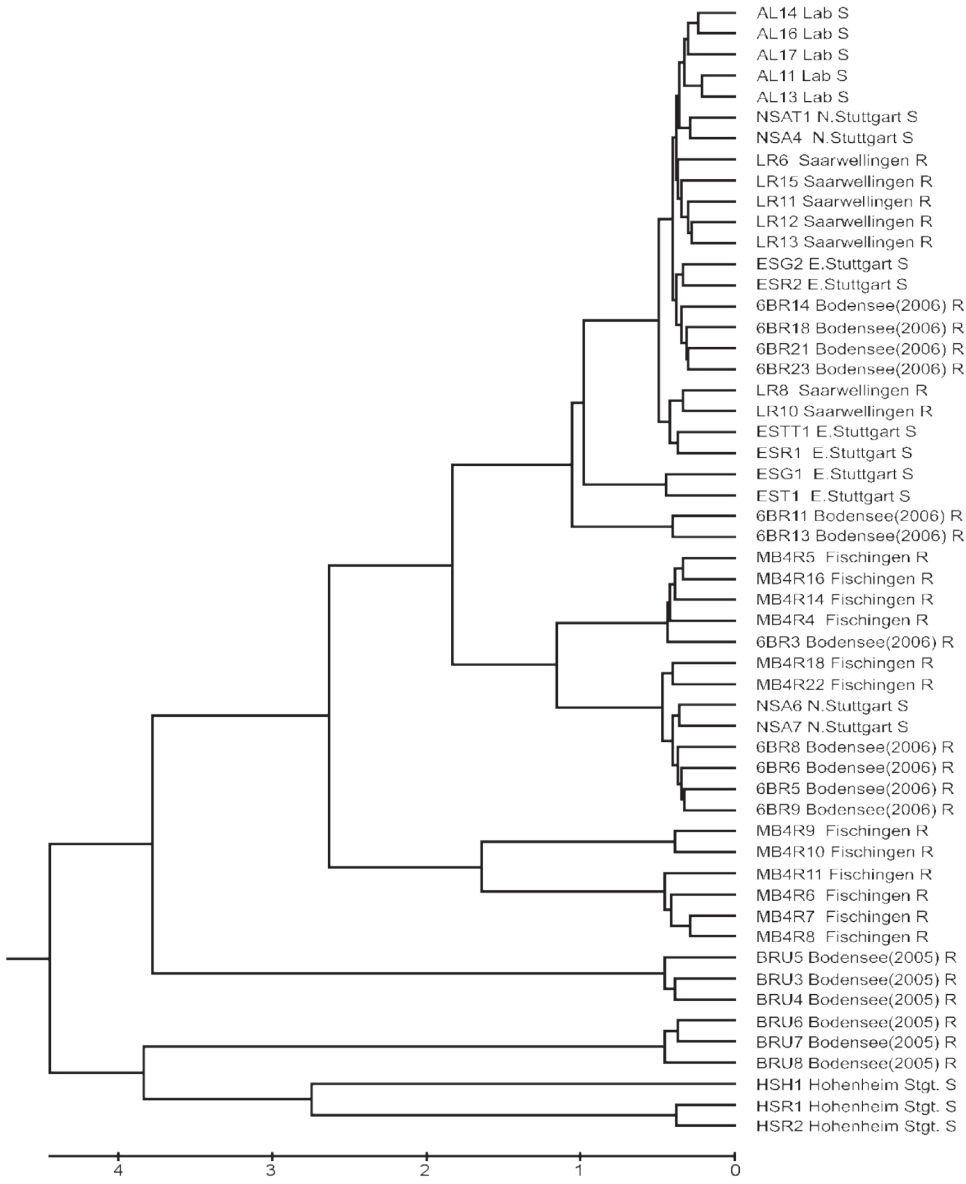

Figure 1. Composite dendrogram generated from 8 different populations and 3 primer combinations using UPGMA method with the program Mega, version 3.0. R = resistant, S = susceptible.

Table 2: Population data [Lynch & Milligan method]

Location	n	Loci P.	P %	Hj ±S.E.	Total No. Fragments	Mean No. Fragments	No. of Segregating Fragments
6BR BWFN	15	171	88.1	0.2897 ± 0.0102	194	60.6	170 (87.6%)
BRU BWFN	6	194	100.0	0.3508 ± 0.0104	194	68.7	142 (73.2%)
MB4R BWFI	14	181	93.3	0.3071 ± 0.0100	194	63.3	181 (93.3%)
LR SLSA	8	194	100.0	0.3047 ± 0.0113	194	59.9	135 (69.6%)
HS BWSH	5	194	100.0	0.3444 ± 0.0098	194	63.4	128 (66.0%)
NS BWSN	7	194	100.0	0.2958 ± 0.0101	194	50.7	140 (72.2%)
ES BWSO	6	194	100.0	0.3231 ± 0.0102	194	61.8	130 (67.8)
AL Lab	6	194	100.0	0.2884 ± 0.0102	194	45.5	115 (59.3%)

n = number of individuals; Loci P. = number of polymorphic loci at the 5% level; P% = Proportion (in %) of polymorphic loci at 5% level; Hj = Nei's gene diversity; S.E. = standard error.

Table 3: Genetic structures of *C. pomonella* populations [Lynch & Milligan method]

Populations Sampled	n	Ht ± S.E.	Hw ± S.E.	Hb± S.E.	Fst
All populations, all individuals (including outgroup)	68	0.4543 ± 0.0007	0.4921 ± 0.0005	-0.0378 ± 0.01456	-0.0831
All populations, all individuals (No outgroup)	67	0.4546 ± 0.0007	0.4923 ± 0.0005	-0.0378 ± 0.01474	-0.0831
All locations	8	0.3300 ± 0.0085	0.3130 ± 0.0022	0.0170 ± 0.1106	0.0516
All resistant	4	0.3312 ± 0.0132	0.3131 ± 0.0023	0.0181 ± 0.1112	0.0548
All susceptible	4	0.3299 ± 0.0129	0.3129 ± 0.0026	0.0170 ± 0.1282	0.0517
Resistant vs. susceptible	2	0.2779 ± 0.0102	0.2743 ± 0.0000	0.0036 ± 0.0365	0.0129
Resistant vs. susceptible wild	2	0.2872 ± 0.0012	0.2833 ± 0.0000	0.0040 ± 0.0042	0.0139
Resistant vs. lab	2	0.2901 ± 0.0019	0.2864 ± 0.0000	0.0037 ± 0.0067	0.0126
Wild vs. laboratory	2	0.2896 ± 0.0032	0.2852 ± 0.0000	0.0044 ± 0.0109	0.0152

n = number of locations; Ht = total gene diversity; Hw = mean gene diversity within a population (Nei's Hs); Hb = average gene diversity (Nei's Dst); Fst = Wright's fixation index; S.E. = standard error.

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