diversity

MDPI

Article

Genetic Diversity in Apple Fruit Moth Indicate Different Clusters in the Two Most Important Apple Growing Regions of Norway

Abdelhameed Elameen *, Hans Geir Eiken and Geir K. Knudsen

NIBIO—Norwegian Institute of Bioeconomy Research, Hogskoleveien 7, N-1432 Aas, Norway; hansgeir.eiken@nibio.no (H.G.E.); geir.knudsen@nibio.no (G.K.K.)

* Correspondence: abdelhameed.elameen@nibio.no; Tel.: +47-902-00875

Academic Editor: Michael Wink

Received: 3 November 2015; Accepted: 6 April 2016; Published: 13 April 2016

Abstract: The apple fruit moth (*Argyresthia conjugella* (*A. conjugella*)) in Norway was first identified as a pest in apple production in 1899. We here report the first genetic analysis of *A. conjugella* using molecular markers. Amplified fragment length polymorphism (AFLP) analysis was applied to 95 individuals from six different locations in the two most important apple-growing regions of Norway. Five AFLP primer combinations gave 410 clear polymorphic bands that distinguished all the individuals. Further genetic analysis using the Dice coefficient, Principal Coordinate analysis (PCO) and Bayesian analyses suggested clustering of the individuals into two main groups showing substantial genetic distance. Analysis of molecular variance (AMOVA) revealed greater variation among populations (77.94%) than within populations (22.06%) and significant and high F_{ST} values were determined between the two major regions (Distance = 230 km, F_{ST} = 0.780). AFLP analysis revealed low to moderate genetic diversity in our population sample from Norway (Average: 0.31 expected heterozygosity). The positive significant correlation between the geographic and the molecular data (r^2 = 0.6700) indicate that genetic differences between the two major regions may be due to geographical barriers such as high mountain plateaus (Hardangervidda) in addition to isolation by distance (IBD).

Keywords: AFLP; *Argyresthia conjugella*; gene flow; genetic differentiation; isolation by distance (IBD); Lepidoptera

1. Introduction

The apple fruit moth (*Argyresthia conjugella* (*A. conjugella*)) was first described by Zeller in 1839. It is a small moth with a body length of 5–6 mm belonging to the order Lepidoptera (butterflies and moths), the group Ditrysia and the family Yponomeutidae. There are at present over 160,000 described species of butterflies and moths [1]. The Ditrysia, a natural group of butterflies and moths, contain more than 98% of the described Lepidoptera species. The *A. conjugella* is a specialized seed predator of rowan (*Sorbus aucuparia*). Females are attracted to unripe rowan berries and lay their eggs on or near clusters of berries. The larvae bore into the fruits where they live and forage. In the late summer, the larvae drop to the ground and pupate. The pupae overwinter in the ground and the adults emerge in May–June the following year. *A. conjugella* has a wide distribution and is found all over Europe, in Asia [2] and have also been reported in North America [3]. In Norway, the *A. conjugella* was first identified as a pest insect in 1899 after a devastating attack on apples [4]. In years when rowan berries are scarce, the female moth must seek alternative hosts [5], and apple (*Malus domestica*) is such a forced alternative host. In years of low rowan fruit production, *A. conjugella* larvae can cause great damage to apple crops, and are therefore recognized as the most important insect pest on apples in Fennoscandia [6].

Genetic diversity is influenced by geographical barriers, dispersal and isolation by distance (IBD). Some insects move only short distances during their entire lifetime [7–9], whereas others engage in one or more bouts of long-distance movement as adults [10,11]. Insect populations may become genetically distinct because of reproductive isolation or lack of gene flow between different regions [12]. Further, reproductive isolation is influenced by several factors such as individual dispersal ability, ecological isolation, geographic barriers, and local adaptation after natural selection [13–16]. Dispersal is a key factor in population dynamics [17]. Continuous dispersal helps long-term survival of a species and can lower the genetic drift rate of local populations [18]. Population genetic studies have shown how genetic differentiation can exist between insecticide-resistant populations and susceptible populations [19,20]. Thus, this may be a key factor for successful and stable control of insect pests. Investigating the genetic diversity of *A. conjugella* may be helpful both in determining sources from which *A. conjugella* disperse and for monitoring insecticide resistance.

Amplified fragment length polymorphism (AFLP) analysis [21] has been widely used to study genetic diversity of plant populations, but only more recently been adopted for population genetic studies of insect species. As an example, AFLP analysis was used to study genetic structure of *Spodoptera frugiperda* populations associated with maize and cotton crops in Brazil [22]. In USA, the technique was applied to detect; high gene flow in *Ostrinia nubilalis* collected from maize fields [23], significant genetic differentiation of *Podisus maculiventris* populations, which is an economical important biological control agent [24] and high genetic variation among regions of the important pest *Pseudatomoscelis seriatus* from cotton fields [25]. In Spain, backcross hybrid between *Drosophila buzzatii* and *Drosophila koepferae* species was detected using AFLP [26]. AFLP was used to detect the impacts of human activities on the Bamboo Locust *Ceracris kiangsu* genetic structure in China [13] and the population dynamics of *Hydropsyche orientalis* and *Stenopsyche marmorata* in Japan [27].

The greatest advantage of AFLP is probably that it does not need previous sequence information of the species. Additional arguments for the choice of AFLP, was the difficulties of the development of microsatellite DNA markers for identification and application of Lepidoptera species, due to the high similarity in flanking regions between different microsatellites within the same species [28–30] as well as the lack of conserved flanking regions leading to unrepeatable banding patterns [31].

AFLP analyses have been used to study the genetic diversity of various insect species, but until now population genetics on *A. conjugella* have not been investigated. Thus, we have used AFLP markers to investigate the genetic differentiation between *A. conjugella* populations in the most important apple growing regions of Southern Norway. The major aim of our study was to investigate whether or not (and to what degree) the potentially strong dispersal barriers between the regions in the form of the high mountain plateau of Hardangevidda, in addition to the geographical distance would limit the gene flow between populations.

2. Materials and Methods

2.1. Collection of A. conjugella Materials

Rowan berries infested with *A. conjugella* larvae were collected in the field during August 2012. Six populations of *A. conjugella* were collected from rowan berries (the primary and preferred host) from two geographical regions (Figure 1). West Norway (1. Espe, 2. Sekse and 3. Stana) and East Norway (4. Fagerstrand, 5. Storsand and 6. Telemark), representing the most important apple growing regions of Norway. The distance between the two major apple growing regions is 230 km, separated by a high mountain plateau (Hardangervidda, see Figure 1). In the east, Telemark sampling location is separated by 90 km from Fagerstrand and 80 km from Storsand, while the distances between the three sampling locations in the west (Espe, Sekse and Stana) are not longer than 5 km (illustrated in Figure 1). For this reason, it was not possible to conduct continuous sampling between the two major regions. Collected *A. conjugella* individuals were stored in 95% ETOH and stored at −20 °C to avoid DNA degradation. Initially, 16 individual insects (8 female and 8 male) were sampled from

each population. One individual female from the location Espe was excluded from the analysis due to lack of PCR amplification. The total number of individuals analyzed was thus 95. Details of the *A. conjugella* individuals are presented in Table 1 and Supplementary Table S1.

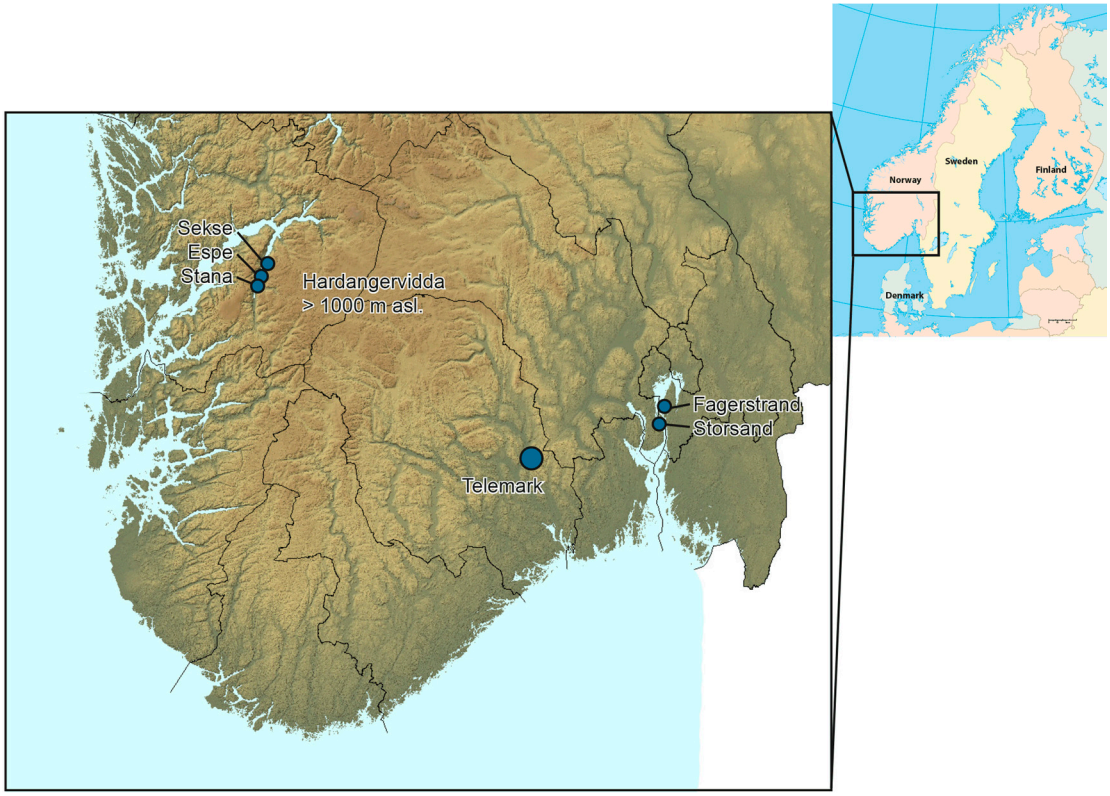

Figure 1. The geographical locations of the sampling areas (blue dots; the size of the dot relative to the size of the sampling area).

Table 1. Overview of *A. conjugella* samples from Southern Norway, n = is the number individuals.

Region	Location	♂	♀	N
West	Espe	8	7	15
	Sekse	8	8	16
	Stana	8	8	16
	N	24	23	47
East	Storsand	8	8	16
	Fagerstrand	8	8	16
	Telemark	8	8	16
	N	24	24	48
Total		48	47	95

2.2. DNA Extraction

DNA was extracted from pupal tissue using DNeasy 96 Blood and Tissue Kits (Qiagen, Tokyo) according to the manufacturer's instructions, and the only modification was the elution of 40 µL DNA instead of 50–100 µL.

2.3. AFLP Analysis

AFLP analysis [21] was performed as previously described [32], with modifications that included the use of fluorescently labeled primers instead of radioactive labeling. Genomic DNA, (approximately

300 ng) was double-digested using *Eco*RI and the *Mse*I isoschizomer *Tru*1I, followed by ligation to the adaptors overnight at room temperature. A total volume of 25 µL pre-amplification containing 5 µL of fivefold diluted ligation product, was carried out with non-selective primers, mixed with; 1× PCR buffer (50 mM KCl, 10 mM Tris HCl pH 8.3, 1.5 mM $MgCl_2$), 600 µM of each dNTP, 50 ng of each 0-primer, and 1 U of Taq DNA polymerase. Pre-amplification was performed in a Gene Amp 9700 thermo cycler (Applied Biosystems, Foster City, CA, USA) as follows: 94 °C for 2 min followed by 45 cycles at 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min, and finally a hold at 72 °C for 10 min.

Eight PCR amplification primer pairs were tested using 10 individuals of *A. conjugella* (both female and male), and of these five pairs with two selective bases (Table 1; Applied Biosystems, Foster City and Invitrogen, Carlsbad, CA, USA) were chosen based on number of amplified fragments in the range 50–500 base pairs, and amount of polymorphism among the included individuals. In the selective PCR amplification, the EcoRI primers are labeled with the fluorescent label 6FAM. The fluorescently labelled PCR products were analyzed using an ABI3730 DNA Analyzer. 1 µL of PCR products were added to a loading buffer containing 8.75 µL Hi-Di formamide (Applied Biosystems), and 0.25 µL of GeneScan 500 LIZ size standard (Applied Biosystems). The data was collected using the software Data Collection v 2.0 (Applied Biosystems), while GeneMapper v 4.1 (Applied Biosystems) was used to derive the fragment length of the labeled DNA-fragments using the known fragment lengths of the LIZ-labeled marker peaks (Supplementary Figure S2).

2.4. Data Analyses

The number of amplified fragments in the range of 50–380 base pairs was recorded manually, and only clear polymorphic bands were scored for presence (1) or absence (0), and monomorphic bands were not scored. The results of the AFLP analysis were confirmed by repeating the analyses of 10 randomly selected *A. conjugella* (both female and male), using same primer combinations. The replicated profiles were compared, and markers with more than 5% errors were removed from the datasets. Genetic similarity (GS) using the Dice coefficient was calculated as $GS_{xy} = 2a/(2a + b + c)$, where GS_{xy} is the measurement of the genetic similarity between insects x and y, a is the number of polymorphic bands present in both insects, b is the number of polymorphic bands present only in insect x, and c is the number of polymorphic bands present only in insect y [33]. The genetic similarity between the different insects, based on presence or absence of the amplified fragments, was also calculated by Jaccard coefficients [34].

Principal Coordinate Analysis (PCO) analyses based on individual insects were performed using four different similarity or distance coefficients (Dice, Simple matching, Jaccard, and Euclidean distance). We used PCO to perform ordination analysis and to classify and detect structure in the relationships between the regions. Statistical analyses were performed using the NTSYS-pc software [35].

We investigated also the genetic structure of *A. conjugella* populations using the model-based Bayesian clustering approach of genetic mixture analysis in the software STRUCTURE 2.3.4 developed by [36], and further implemented by [37]. These analyses assume that the clusters (K) are at Hardy–Weinberg equilibrium and in linkage equilibrium, but the approach has proven to be robust also to deviations from these assumptions [37]. Simulations were performed with data set, from K = 1 to K = 6. Six independent runs were conducted to assess the consistency of the results across runs, and all runs were based on 500,000 iterations after a burn-in period of 100,000 iterations. We followed the method developed by [38], to identify the number of genetically homogeneous clusters (K). Plots of likelihoods, similarity coefficients and ΔKs [38] were made with Structure Harvester [39].

Analysis of molecular variance (AMOVA) [40] for *A. conjugella* populations was used to estimate the partitioning of AFLP variation between the regions and among populations within different regions. We also performed a separate AMOVA on the Western populations (Supplementary Table S2A) and a separate one on the Eastern populations (Supplementary Table S2B).

The genetic distances among all *A. conjugella* populations were calculated using pair-wise genetic distance method [41]. The F_{ST} values and mean F_{ST} was estimated in order to study genetic differentiation between regions and among local populations. The significance of F_{ST} values was tested by 1000 permutation. These analyses were performed using Arlequin software, version 2.000 [42]. Gene flow was estimated assuming $Nm = (1/F_{ST} - 1)/4$ [43].

The geographic data (based on the distance between the Eastern and the Western populations) were compared and correlated with the molecular data (AFLP analyses), the correlations were estimated using Mantel's test [44].

3. Results

AFLP analyses of all 95 *A. conjugella* using five primer combinations resulted in a total number of 410 clear polymorphic bands (Table 2). The number of polymorphic bands per primer combination ranged from 75 to 91 with an average of 82 polymorphic bands per primer pair. Using these five primer combinations, it was possible to distinguish between all 95 *A. conjugella* individuals.

Table 2. Sequences of the *Eco*RI and *Mse*I selective primers used for AFLP analysis.

Primer Combination	EcoRI Primer 5′-3′	MseI Primer 5′-3′	Number of Polymorphic Markers
$EcoRI_0 \times MseI_0$	GACTGCGTACCAATTC	GATGAGTCCTGAGTAA	
$EcoRI_{19} \times MseI_{15}$	6FAM-GACTGCGTACCAATTCGA	GATGAGTCCTGAGTAACA	77
$EcoRI_{19} \times MseI_{16}$	6FAM-GACTGCGTACCAATTCGA	GATGAGTCCTGAGTAACC	86
$EcoRI_{19} \times MseI_{17}$	6FAM-GACTGCGTACCAATTCGA	GATGAGTCCTGAGTAACG	75
$EcoRI_{12} \times MseI_{16}$	6FAM-GACTGCGTACCAATTCAC	GATGAGTCCTGAGTAACC	91
$EcoRI_{12} \times MseI_{17}$	6FAM-GACTGCGTACCAATTCAC	GATGAGTCCTGAGTAACG	81

The results of the principal coordinate analysis (PCO) clustered the 95 *A. conjugella* into two main groups. PCO based on alternative coefficients (see Methods) gave very similar results as above (Results not shown). There is a clear distinguishable pattern of clustering without overlapping of *A. conjugella* from the two major regions (Figure 2).

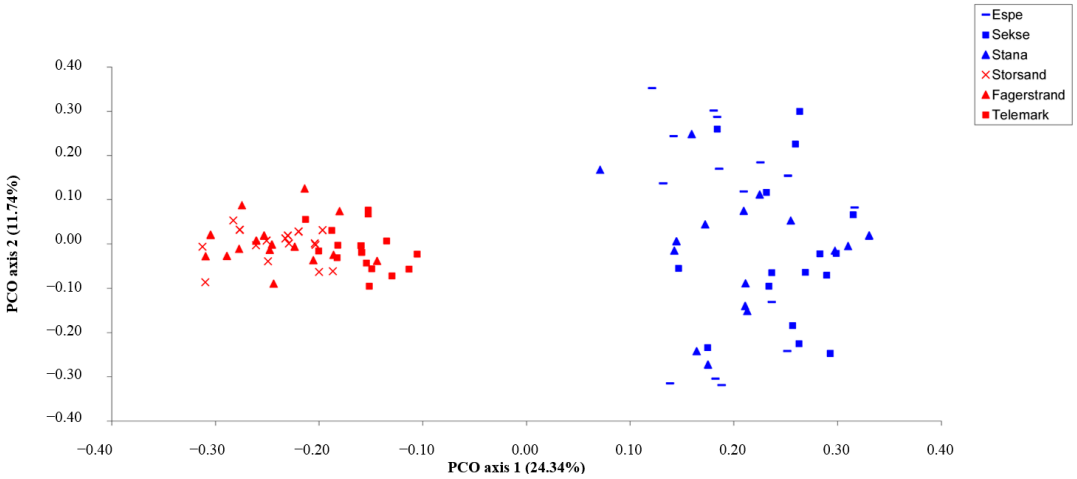

Figure 2. PCO score plot of 95 *A. conjugella* individuals using 410 polymorphic AFLP bands. The geographical origin of each *A. conjugella* individual is indicated by the symbols listed at the right; red: Eastern region and blue: Western region (Eigenvalue for each axis is given in brackets).

Structure analysis showed the maximum likelihood distribution *L (K)* at the real number of two groups (K = 2) (Figure 3 and Supplementary Figure S1). This value was obtained, using the value of *Ad hoc* quantity (ΔK) rather than maximum likelihood value *L (K)* as published by [38]. Structure analysis clustered the *A. conjugella* into two main clusters supported the results of the PCO analysis. There is a clear distinguishable pattern of clustering of *A. conjugella* from the two major regions. Both clustering analyses showed that individual males and females from both regions were clustered randomly, and there was no distinguishable pattern of clustering of *A. conjugella* according to their sex.

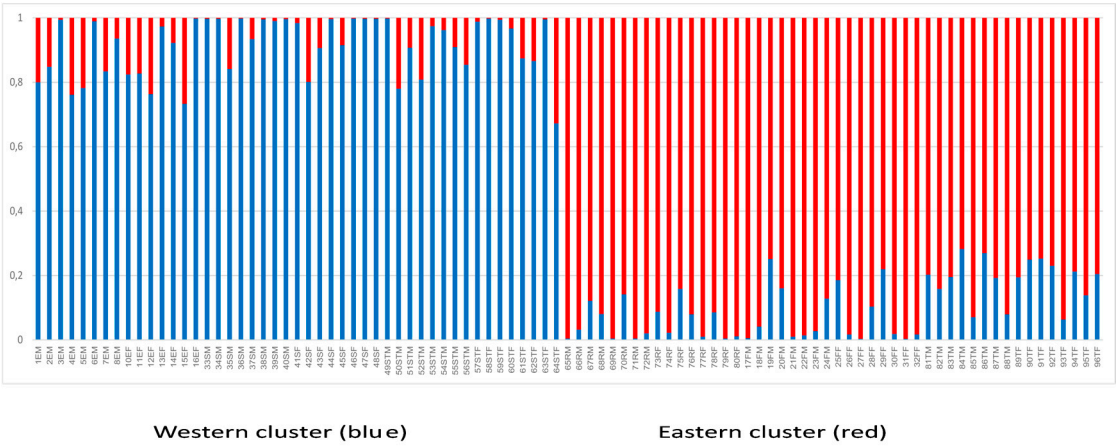

Figure 3. Population genetics structure analyses of all 95 individuals in the study using STRUCTURE software program. The insects are sorted according to Id number as in Supplementary Table S1 (blue: Western cluster and red: Eastern cluster).

The AFLP data were also analyzed using AMOVA, and variance components were estimated (Table 3). Most of the total genetic variability in *A. conjugella* (77.94%) was among regions, while the genetic variability within regions and among populations within regions was low (21.20% and 4.73%) respectively. In contrast, AMOVA analysis of the Western populations (Supplementary Table S2) and the Eastern populations (Supplementary Table S3), showed high genetic variability within populations and low genetic variability among populations, as well as low F_{ST} values 0.0078 and 0.1053 respectively.

Table 3. Analysis of molecular variance (AMOVA) of 95 *A. conjugella* between the two major (East and west) regions using 410 AFLP markers.

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Within regions	1	898.083	15.79340	17.33
Among populations with regions	3	560.000	4.31102	4.73
Among regions	90	6392.125	71.02361	77.94
Total	94	7850.208	91.12804	

The analysis of pair-wise distances showed that the genetic distances between *A. conjugella* locations in the Eastern and Western clusters were high (Table 4). The greatest genetic distance between populations was between Fagerstrand in the Eastern region and Sekse in the Western region (0.26121), while the smallest genetic distance was between Espe and Stana, and both are from the Western region (0.01469).

Table 4. Pair-wise F_{ST} values among the six sampling locations of *A. conjugella* in Southern Norway.

Regions	1	2	3	4	5	6
1	0.00000					
2	0.01469	0.00000				
3	0.00193	0.00496	0.00000			
4	0.19706	0.26121	0.22103	0.00000		
5	0.19280	0.26079	0.22350	0.01826	0.00000	
6	0.17872	0.24820	0.20326	0.13837	0.14781	0.00000

Western (1. Espe, 2. Sekse and 3. Stana) and Eastern (4. Fagerstrand, 5. Storsand and 6. Telemark).

The mean expected heterozygosity (0.306), and the level of expected heterozygosity in the Western populations (0.335) was higher than in the Eastern populations (0.276). The F_{ST} value between the two major regions is high (0.780), while the F_{ST} values between the three sampling locations within the Western and Eastern regions were very low to moderate, respectively (Table 5). The gene flow (Nm) was estimated to be very low (0.40) between the two major regions, while within Western and Eastern regions gene flow was found to be very high and moderate, respectively (Table 5).

Table 5. Summary of the genetic diversity analyses of *A. conjugella* in Southern Norway based on 410 AFLP polymorphic bands.

Regions (No)	No. of Individuals	Expected Heterozygosity	Gene Flow (Nm)	Mean F_{ST}
Western (1, 2 and 3)	47	0.335	34.80	0.007
Eastern (4, 5 and 6)	48	0.276	2.37	0.105
The mean and the total	95	0.306	0.40	0.780

Western (1. Espe, 2. Sekse and 3. Stana) and Eastern (4. Fagerstrand, 5. Storsand and 6. Telemark).

Mantel test showed a positive significant correlation ($r^2 = 0.6700$) between the geographic and the molecular data ($p \leqslant 0.001$).

4. Discussion

To our knowledge, this the first study that investigates genetic diversity of *A. conjugella* using molecular markers, and it is the first evidence of geographic variation in *A. conjugella* populations in Norway. We detected low to moderate genetic diversity in *A. conjugella* populations, and found very high genetic differentiation between the two most important apple growing regions in Norway. Gene flow in insect species is expected to be high in populations that have no major geographical barriers and is not separated by long distances [45]. Although the distance between the two major apple growing regions in Southern Norway is only 230 km, these two major areas are separated by a high mountain plateau (Hardangervidda), which is more than 1000 m above the sea level. For this reason, continuous sampling between the two regions was not possible. Thus, substantial genetic differentiation may be expected as a result of the long distance and physical barriers that both may strongly limit gene flow.

All our genetic analyses show that most of the genetic variation and structuring was between the Eastern and the Western regions in Southern Norway. The pairwise F_{ST} analysis also indicated substantial geographical structuring, showing a significant and very high F_{ST} value between these two major regions (0.780). Due to the lack of previous genetics studies in *A. conjugella*, we compared this F_{ST} result to other studies in Lepidoptera species. A comparable high value of 0.708 in *Busseola fusca* was obtained in a geographical wide study among Eastern, Central and Western African countries [46] where the regions are separated by more than 10 times the distance of our study (3000 km). A more moderate value of 0.437 in *Tuta absoluta* [47] was found between two regions in Tunisia separated by 170 km that may be more comparable to our study. The very high value of 0.90 in *Boloria eunomia* [48]

was detected among Western, Central and southern Europe, and this is not surprising since these regions are separated by more than 2500 km. Thus, our F_{ST} value may be regarded as very high, which indicates that structuring and differentiation between regions may be influenced by more than IBD alone. Significant correlations between genetic and geographic distances using Mantel's test supported these results. Our results may be comparable to the ones obtained for other Lepidoptera species, e.g., "*Sesamia nonagrioides*" in Iran and Spain where subpopulations are separated by larger distances than in the present study. In Iran, genetic differentiation was found between two regions (Southern and Southwestern Iran) separated by 500 km [49], while in Spain, high genetic differentiation was observed between regions separated by distances of 1000 km [50]. AFLP data analysis of individual male and female did not reveal any marker specific for sex determination, and clustering analyses of males and females did not show any particular pattern. This result is similar to the one obtained in the Lepidoptera species "*Plutella xylostella*" [51]. For the same species, in Cameroon and China substantial genetic variation and high gene flow among regions have been reported [52,53]. However, recent studies also provide contrasting results and have reported genetic differentiation and isolated populations in Lepidoptera species such as for *Boloria eunomia*, *Sesamia nonagriodes* and *Diatraea saccharalis* [48,49,54]. Our results may be an example of a combination of geographical barriers and IBD causing very high genetic differentiation between regions. In addition, moderate to high gene flow was observed among populations within each of the two major region in Southern Norway, indicating that *A. conjugella* show medium to short distance migration. Similarly, the cotton flea-hopper's (*Hemiptera: Miridae*) western populations in USA are genetically distinct from eastern populations, but with considerable gene flow among the populations within each region [25]. Gene flow in the west indicate a total contact between the three sampling locations probably as a result of very small geographical distances, while in the East the gene flow value (~2) may indicate some structuring among the three sampling locations maybe as a result of longer distances and IBD.

There are several other possible factors, which might be responsible for genetic differentiation among populations in insects species; low dispersal ability [15,55], habitat fragmentation [56–58] and host plant availability [6,55,59]. *A. conjugella* in different regions could be genetically distinct, considering the differences in eco-geographic factors and variable presence of this insect in the rowan and apple plants in Norway. Furthermore, variable rates of natural selection may act on species with a wide geographic distribution and therefore, locally adapted populations will exist, which will result in high genetic variation among regions. Genetic differentiation between the two regions may also be due to two different introductions *A. conjugella* in Norway, but we do not have enough data to investigate this scenario and further investigation is therefore also required.

A. conjugella analyses in the study showed a lower expected heterozygosity (He = 0.31), than was found in various insect species such as; *Metrioptera roeselii* "0.61" [60]; *Leptomyrmex pallens* "0.51" [61] and *Cerambyx cerdo* "0.63" [62]. However, the low expected heterozygosity was comparable to what was found in *Plutella xylostella* "0.35" [11], *Anopheles nuneztovari* (Culicidae) "0.34" [63], *Glossina pallidipes* "0.35" [64] and *Bombus distinguendus* "0.38" [65]. The regionally synchronous and variable seed set of rowan, also known as masting, will have a great impact on *A. conjugella* as it is a seed predator. The masting in rowan may explain the low level of heterozygosity in *A. conjugella*. In Norway, it was suggested that the masting in rowan is an example of defence against seed predation by the process of predator satiation [6]. In intermast years, the low berry production will inhibit and strongly influence seed predator population size. In the next masting year, rowan takes advantage of the reduced number of seed predators and produce a high crop with low losses to predators. The low level of expected heterozygosity may be caused by intermast years resulting in repetitive bottlenecks. Bottleneck processes may also have been caused by historically intensive use of insecticides to control *A. conjugella*, which have caused a selective pressure.

In conclusion, our study indicates substantial genetic differentiation and low genetic variation for the species in a restricted area in Southern Norway. However, we believe these findings should be investigated further in a much larger area, so we have identified multiple STRs (not shown), and we

intend to increase both the geographical area and the sample size. Increased knowledge on genetic factors might in the future lead to a more efficient management of *A. conjugella* as an important pest insect in apple production.

Supplementary Materials: The supplementary materials are available online at www.mdpi.com/1424-2818/8/2/10/s1.

Acknowledgments: This work was financed by Norwegian Institute for Bioeconomy Research. The authors would like to thank Sverre Kobro for sampling the collections of *A. conjugella*.

Author Contributions: Abdelhameed Elameen, Hans Geir Eiken and Geir K. Knudsen designed and conceived the study; Abdelhameed Elameen and Hans Geir Eiken developed the methods; Analyses were performed by Abdelhameed Elameen and Hans Geir Eiken. The manuscript was written by Abdelhameed Elameen and Hans Geir Eiken with input from Geir K. Knudsen.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kristensen, N.P.; Scoble, M.J.; Karsholt, O. Lepidoptera phylogeny and systematics: The state of inventorying moth and butterfly diversity. *Zootaxa* **2007**, *1668*, 699–747.
2. Sharma, J.; Khajuria, D.; Dogra, G. Studies on the apple fruit moth, *Argyresthia conjugella* zeller (Yponomeutidae: Lepidoptera): Identification, distribution and extent of damage in India. *Int. J. Pest Manag.* **1988**, *34*, 189–192.
3. Fletcher, J. *Canada, Department of Agriculture, Central Experimental Farm. Report of the Entomologist and Botanist 1896*; Government Printing Bureau: Ottawa, ON, Canada, 1897; pp. 226–276.
4. Schøyen, T.H. *Beretning om skadeinsekter og Plantesygdomme*; Grøndahl og Søns bogtrykkeri: Kristiania, Norway, 1899. (In Norwegian)
5. Bengtsson, M.; Jaastad, G.; Knudsen, G.; Kobro, S.; Bäckman, A.C.; Pettersson, E.; Witzgall, P. Plant volatiles mediate attraction to host and non-host plant in apple fruit moth, *Argyresthia conjugella*. *Entomol. Exp. Appl.* **2006**, *118*, 77–85. [CrossRef]
6. Kobro, S.; Søreide, L.; Djønne, E.; Rafoss, T.; Jaastad, G.; Witzgall, P. Masting of rowan *Sorbus aucuparia* L. and consequences for the apple fruit moth *Argyresthia conjugella* zeller. *Popul. Ecol.* **2003**, *45*, 25–30.
7. Ribeiro, D.B.; Batista, R.; Prado, P.I.; Brown, K.S., Jr.; Freitas, A.V. The importance of small scales to the fruit-feeding butterfly assemblages in a fragmented landscape. *Biodivers. Conserv.* **2012**, *21*, 811–827. [CrossRef]
8. Tufto, J.; Lande, R.; Ringsby, T.H.; Engen, S.; Sæther, B.E.; Walla, T.R.; DeVries, P.J. Estimating brownian motion dispersal rate, longevity and population density from spatially explicit mark–recapture data on tropical butterflies. *J. Anim. Ecol.* **2012**, *81*, 756–769. [CrossRef] [PubMed]
9. Legal, L.; Dorado, O.; Machkour-M'Rabet, S.; Leberger, R.; Albre, J.; Mariano, N.A.; Gers, C. Ecological constraints and distribution of the primitive and enigmatic endemic mexican butterfly *baronia brevicornis* (lepidoptera: Papilionidae). *Can. Entomol.* **2015**, *147*, 71–88. [CrossRef]
10. Dingle, H.; Drake, V.A. What is migration? *Bioscience* **2007**, *57*, 113–121. [CrossRef]
11. Roux, O.; Gevrey, M.; Arvanitakis, L.; Gers, C.; Bordat, D.; Legal, L. ISSR: Tool for discrimination and genetic stucture analyses of *Plutella xylostella* populations native to different geographical areas. *Mol. Phylogenet. Evol.* **2007**, *43*, 240–250. [CrossRef] [PubMed]
12. Laffin, R.; Langor, D.; Sperling, F. Population structure and gene flow in the white pine weevil, *Pissodes strobi* (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Am.* **2004**, *97*, 949–956. [CrossRef]
13. Fan, Z.; Jiang, G.F.; Liu, Y.X.; He, Q.X.; Blanchard, B. Population explosion in the yellow-spined bamboo Locust *Ceracris kiangsu* and inferences for the impact of human activity. *PLoS ONE* **2014**, *9*, e89873. [CrossRef] [PubMed]
14. Gomez, A. Molecular ecology of rotifers: From population differentiation to speciation. *Hydrobiologia* **2005**, *546*, 83–99. [CrossRef]
15. Margaritopoulos, J.T.; Voudouris, C.C.; Olivares, J.; Sauphanor, B.; Mamuris, Z.; Tsitsipis, J.A.; Franck, P. Dispersal ability in codling moth: Mark–release–recapture experiments and kinship analysis. *Agric. For. Entomol.* **2012**, *14*, 399–407. [CrossRef]

16. Voudouris, C.C.; Franck, P.; Olivares, J.; Sauphanor, B.; Mamuris, Z.; Tsitsipis, J.; Margaritopoulos, J. Comparing the genetic structure of codling moth *Cydia pomonella* (L.) from Greece and France: Long distance gene-flow in a sedentary pest species. *Bull. Entomol. Res.* **2012**, *102*, 185–198. [CrossRef] [PubMed]
17. Azevedo, F.; Coutinho, R.; Kraenkel, R. Spatial dynamics of a population with stage-dependent diffusion. *Commun. Nonlinear Sci. Numer. Simul.* **2015**, *22*, 605–610. [CrossRef]
18. Kaňuch, P.; Berggren, Å.; Cassel-Lundhagen, A. Colonization history of *Metrioptera roeselii* in Northern Europe indicates human-mediated dispersal. *J. Biogeogr.* **2013**, *40*, 977–987. [CrossRef]
19. Chen, H.; Wang, H.; Siegfried, B.D. Genetic differentiation of Western corn rootworm populations (*Coleoptera*: Chrysomelidae) relative to insecticide resistance. *Ann. Entomol. Soc. Am.* **2012**, *105*, 232–240. [CrossRef]
20. Franck, P.; Reyes, M.; Olivares, J.; Sauphanor, B. Genetic architecture in codling moth populations: Comparison between microsatellite and insecticide resistance markers. *Mol. Ecol.* **2007**, *16*, 3554–3564. [CrossRef] [PubMed]
21. Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; van De Lee, T.; Hornes, M.; Friters, A.; Pot, J.; Paleman, J.; Kuiper, M. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* **1995**, *23*, 4407–4414. [CrossRef] [PubMed]
22. Martinelli, S.; Clark, P.; Zucchi, M.; Silva-Filho, M.; Foster, J.E.; Omoto, C. Genetic structure and molecular variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in maize and cotton fields in Brazil. *Bull. Entomol. Res.* **2007**, *97*, 225–231. [CrossRef] [PubMed]
23. Krumm, J.T.; Hunt, T.E.; Skoda, S.R.; Hein, G.L.; Lee, D.J.; Clark, P.L.; Foster, J.E. Genetic variability of the European corn borer, *Ostrinia nubilalis*, suggests gene flow between populations in the Midwestern United States. *J. Insect Sci.* **2008**, *8*, 72. [CrossRef] [PubMed]
24. Kneeland, K.; Coudron, T.A.; Lindroth, E.; Stanley, D.; Foster, J.E. Genetic variation in field and laboratory populations of the spine soldier bug, *Podisus maculiventris*. *Entomol. Exp. Appl.* **2012**, *143*, 120–126. [CrossRef]
25. Barman, A.K.; Sansone, C.; Parajulee, M.N.; Medina, R.F. Population genetic structure of *Pseudatomoscelis seriatus* (Hemiptera: Miridae) in the cotton-growing regions of the United States. *J. Econ. Entomol.* **2013**, *106*, 2225–2233. [CrossRef] [PubMed]
26. Vela, D.; García Guerreiro, M.P.; Fontdevila, A. Adaptation of the AFLP technique as a new tool to detect genetic instability and transposition in interspecific hybrids. *BioTechniques* **2011**, *50*, 247. [PubMed]
27. Nukazawa, K.; Kazama, S.; Watanabe, K. A hydrothermal simulation approach to modelling spatial patterns of adaptive genetic variation in four stream insects. *J. Biogeogr.* **2015**, *42*, 103–113. [CrossRef]
28. Meglecz, E.; Petenian, F.; Danchin, E.; D'Acier, A.C.; Rasplus, J.Y.; Faure, E. High similarity between flanking regions of different microsatellites detected within each of two species of Lepidoptera: *Parnassius apollo* and *Euphydryas aurinia*. *Mol. Ecol.* **2004**, *13*, 1693–1700. [CrossRef] [PubMed]
29. Meglécz, E.; Solignac, M. Brief report microsatellite loci for *Parnassius mnemosyne* (Lepidoptera). *Hereditas* **1998**, *128*, 179–180. [CrossRef]
30. Zhan, J.; McDonald, B. The interaction among evolutionary forces in the pathogenic fungus *Mycosphaerella graminicola*. *Fung. Genet. Biol.* **2004**, *41*, 590–599. [CrossRef] [PubMed]
31. Sarhan, A. Isolation and characterization of five microsatellite loci in the Glanville fritillary butterfly (*Melitaea cinxia*). *Mol. Ecol. Notes* **2006**, *6*, 163–164. [CrossRef]
32. Elameen, A.; Fjellheim, S.; Larsen, A.; Rognli, O.A.; Sundheim, L.; Msolla, S.; Masumba, E.; Mtunda, K.; Klemsdal, S.S. Analysis of genetic diversity in a sweet potato (*Ipomoea batatas* L.) germplasm collection from Tanzania as revealed by AFLP. *Genet. Resour. Crop Evol.* **2008**, *55*, 397–408. [CrossRef]
33. Dice, L.R. Measures of the amount of ecologic association between species. *Ecology* **1945**, *26*, 297–302. [CrossRef]
34. Jaccard, P. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vand. Sci. Nat.* **1908**, *44*, 223–270.
35. Rohlf, F.J. *NTsys-pc: Numerical Taxonomy and Multivariate Analysis System*; Exeter Publishing: Setauket, NY, USA, 1992.
36. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [PubMed]
37. Falush, D.; Stephens, M.; Pritchard, J.K. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* **2003**, *164*, 1567–1587. [PubMed]
38. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software structure: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef] [PubMed]

39. Earl, D.A. Structure harvester: A website and program for visualizing structure output and implementing the Evanno method. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
40. Excoffier, L.; Smouse, P.E.; Quattro, J.M. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **1992**, *131*, 479–491. [PubMed]
41. Excoffier, L.; Smouse, P.E. Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: Molecular variance parsimony. *Genetics* **1994**, *136*, 343–359. [PubMed]
42. Schneider, S.; Roessli, D.; Excoffier, L. Arlequin: A software for population genetics data analysis. *User Manu. Ver.* **2000**, *2*, 2496–2497.
43. Whitlock, M.C.; McCauley, D.E. Indirect measures of gene flow and migration: $F_{ST} = 1/(4\ nm + 1)$. *Heredity* **1999**, *82*, 117–125. [CrossRef] [PubMed]
44. Mantel, N. The detection of disease clustering and a generalized regression approach. *Cancer Res.* **1967**, *27*, 209–220. [PubMed]
45. Agunbiade, T.A.; Coates, B.S.; Datinon, B.; Djouaka, R.; Sun, W.; Tamo, M.; Pittendrigh, B.R. Genetic differentiation among *Maruca vitrata* f. (Lepidoptera: Crambidae) populations on cultivated cowpea and wild host plants: Implications for insect resistance management and biological control strategies. *PLoS ONE* **2014**, *9*, e92072. [CrossRef] [PubMed]
46. Sezonlin, M.; Dupas, S.; Le Rü, B.; Le Gall, P.; Moyal, P.; Calatayud, P.A.; Giffard, I.; Faure, N.; Silvain, J.F. Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa. *Mol. Ecol.* **2006**, *15*, 407–420. [CrossRef] [PubMed]
47. Bettaïbi, A.; Mezghani-Khemakhem, M.; Bouktila, D.; Makni, H.; Makni, M. Genetic variability of the tomato leaf miner (*Tuta absoluta meyrick*; Lepidoptera: Gelechiidae), in Tunisia, inferred from RAPD-PCR. *Chil. J. Agric. Res.* **2012**, *72*, 212–216. [CrossRef]
48. Legrand, D.; Chaput-Bardy, A.; Turlure, C.; Dubois, Q.; Huet, M.; Schtickzelle, N.; Stevens, V.M.; Baguette, M. Isolation and characterization of 15 microsatellite loci in the specialist butterfly *Boloria eunomia*. *Conserv. Genet. Resour.* **2014**, *6*, 223–227. [CrossRef]
49. Cheraghali, Z.; Esfandiari, M.; Mossadegh, M.S.; Memari, H.R. Genetic diversity of populations of the stem borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae) in southern and southwestern Iran, using RAPD-PCR. *North-West. J. Zool.* **2015**, *11*, 70–75.
50. De La Poza, M.; Farinós, G.P.; Beroiz, B.; Ortego, F.; Hernández-Crespo, P.; Castañera, P. Genetic structure of *Sesamia nonagrioides* (lefebvre) populations in the Mediterranean area. *Environ. Entomol.* **2008**, *37*, 1354–1360. [CrossRef]
51. Pichon, A.; Arvanitakis, L.; Roux, O.; Kirk, A.; Alauzet, C.; Bordat, D.; Legal, L. Genetic differentiation among various populations of the diamondback moth, *Plutella xylostella* Lepidoptera yponomeutidae. *Bull. Entomol. Res.* **2006**, *96*, 137–144. [CrossRef] [PubMed]
52. Sezonlin, M.; Ndemah, R.; Goergen, G.; Le Rü, B.; Dupas, S.; Silvain, J.F. Genetic structure and origin of *Busseola fusca* populations in Cameroon. *Entomol. Exp. Appl.* **2012**, *145*, 143–152. [CrossRef]
53. Wei, S.J.; Shi, B.C.; Gong, Y.J.; Jin, G.H.; Chen, X.X.; Meng, X.F. Genetic structure and demographic history reveal migration of the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) from the southern to northern regions of china. *PLoS ONE* **2013**, *8*, e59654. [CrossRef] [PubMed]
54. Silva-Brandão, K.L.; Santos, T.V.; Cônsoli, F.L.; Omoto, C. Genetic diversity and structure of Brazilian populations of *Diatraea saccharalis* (Lepidoptera: Crambidae): Implications for pest management. *J. Econ. Entomol.* **2015**, *108*, 307–316. [CrossRef] [PubMed]
55. St Pierre, M.J.; Hendrix, S.D.; Lewis, C.K. Dispersal ability and host-plant characteristics influence spatial population structure of monophagous beetles. *Ecol. Entomol.* **2005**, *30*, 105–115. [CrossRef]
56. DeChaine, E.G.; Martin, A.P. Historic cycles of fragmentation and expansion in *Parnassius smintheus* (Papilionidae) inferred using mitochondrial DNA. *Evolution* **2004**, *58*, 113–127. [CrossRef] [PubMed]
57. Meng, X.F.; Shi, M.; Chen, X.X. Population genetic structure of *Chilo suppressalis* (walker) (Lepidoptera: Crambidae): Strong subdivision in china inferred from microsatellite markers and mt-DNA gene sequences. *Mol. Ecol.* **2008**, *17*, 2880–2897. [CrossRef] [PubMed]
58. Seyahooei, M.A.; van Alphen, J.J.; Kraaijeveld, K. Genetic structure of *Leptopilina boulardi* populations from different climatic zones of Iran. *BMC Ecol.* **2011**, *11*, 4. [CrossRef] [PubMed]

59. Mopper, S. Adaptive genetic structure in phytophagous insect populations. *Trends Ecol. Evol.* **1996**, *11*, 235–238. [CrossRef]
60. Kaňuch, P.; Berggren, Å.; Cassel-Lundhagen, A. Genetic diversity of a successful colonizer: Isolated populations of *Metrioptera roeselii* regain variation at an unusually rapid rate. *Ecol. Evol.* **2014**, *4*, 1117–1126. [CrossRef] [PubMed]
61. Berman, M.; Austin, C.M.; Miller, A.D. Characterization of the complete mitochondrial genome and 13 microsatellite loci through next-generation sequencing for the new Caledonian spider-ant *Leptomyrmex pallens*. *Mol. Biol. Rep.* **2014**, *41*, 1179–1187. [CrossRef] [PubMed]
62. Drag, L.; Kosnar, J.; Cizek, L. Development and characterization of ten polymorphic microsatellite loci for the great Capricorn beetle (*Cerambyx cerdo*) (Coleoptera: Cerambycidae). *Conserv. Genet. Resour.* **2013**, *5*, 907–909. [CrossRef]
63. Elisa Posso, C.; González, R.; Cárdenas, H.; Gallego, G.; Duque, M.C.; Suarez, M.F. Random amplified polymorphic DNA analysis of *Anopheles nuneztovari* (Diptera: Culicidae) from western and Northeastern Colombia. *Memórias do Instituto Oswaldo Cruz* **2003**, *98*, 469–476. [CrossRef]
64. Ciosi, M.; Masiga, D.K.; Turner, C.M.; Valenzuela, J.G. Laboratory colonisation and genetic bottlenecks in the tsetse fly *Glossina pallidipes*. *PLoS Negl. Trop. Dis.* **2014**, *8*, e2697. [CrossRef] [PubMed]
65. Charman, T.G.; Sears, J.; Green, R.E.; Bourke, A.F. Conservation genetics, foraging distance and nest density of the scarce great yellow bumblebee (*Bombus distinguendus*). *Mol. Ecol.* **2010**, *19*, 2661–2674. [CrossRef] [PubMed]