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Rust mite resistance in apple assessed by quantitative trait loci analysis

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Abstract The aim of this study was to assess the genetic basis of rust mite (*Aculus schlechtendali*) resistance in apple (*Malus* × *domestica*). *A. schlechtendali* infestation of apple trees has increased as a consequence of reduced side effects of modern fungicides on rust mites. An analysis of quantitative trait loci (QTLs) was carried out using linkage map data available for F_1 progeny plants of the cultivars 'Fiesta' × 'Discovery'. Apple trees representing 160 different genotypes were surveyed for rust mite infestation, each at three different sites in two consecutive years. The distribution of rust mites on the individual apple genotypes was aggregated and significantly affected by apple genotype and site. We identified two QTLs for *A. schlechtendali* resistance on linkage group 7 of 'Fiesta'. The AFLP marker E35M42-0146 (20.2 cM) and the RAPD marker AE10-400 (45.8 cM) were closest positioned to the QTLs and explained between 11.0% and 16.6% of the phenotypic variability. Additionally, putative QTLs on the 'Discovery' chromosomes 4, 5 and 8 were detected. The SSR marker Hi03a10 identified to be associated to one of the QTLs (AFLP marker E35M42-0146) was traced back in the 'Fiesta' pedigree to the apple cultivar 'Wagener'. This marker may facilitate the breeding of resistant apple cultivars by marker assisted selection. Furthermore, the genetic background of rust mite resistance in existing cultivars can be evaluated by testing them for the identified SSR marker.

Keywords Quantitative trait loci · Apple *Malus* × *domestica* · Rust mite resistance

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Introduction

The apple rust mite (*Aculus schlechtendali* Nalepa) is a serious pest in many apple growing regions of the world (Easterbrook and Palmer 1996). *A. schlechtendali* infestation of apple trees (*Malus* × *domestica* Borkh.) has increased along with changes from broad-spectrum to non-acaricidal fungicides (Easterbrook 1984). Many formerly applied sulfur-containing products for disease control and also products for pest control exhibited side effects on rust mites and other non-target organisms, and have now been replaced by selective compounds devoid of such effects (Spieser et al. 1998). High numbers of *A. schlechtendali* cause browning of leaf undersides and early defoliation (Easterbrook and Fuller 1986), which result in a reduced CO_2 exchange and transpiration rate, and negatively affect yield, fruit quality and tree growth (Spieser et al. 1998). In addition, feeding *A. schlechtendali* can initiate russet formation, rendering fruits unmarketable (Easterbrook and Fuller 1986).

The use of resistant cultivars is an important component of integrated pest management (IPM) (Frei et al. 2005),

which combines resistant plants with chemical, biological and cultural control methods to reduce plant damage and minimize pesticide applications (Kellerhals et al. 2004; Mody et al. 2008). The potential influence of the apple cultivar on *A. schlechtendali* resistance has been reported by several studies (Downing and Moilliet 1967; Herbert 1974; Höhn and Höpli 1990; Easterbrook and Palmer 1996; Graf et al. 1998; Spieser et al. 1998; Duso et al. 2003). However, the resistance of these apple cultivars was scored by phenotypic evaluation, and no information on the genetic basis of *A. schlechtendali* resistance is yet available. Consequently, the applicability of such cultivars for resistance breeding in apple is limited. Phenotypic evaluation is either unreliable, or substantial resources are needed for additional, complex field trials (Brown and Maloney 2003; Francia et al. 2005).

Genetic linkage maps allow the identification of quantitative trait loci (QTLs), which can indicate chromosomal regions controlling phenotypic traits (Collard et al. 2005). Such a linkage map should be densely covered with molecular markers, in order to obtain the maximum probability to identify QTLs (Silfverberg-Dilworth et al. 2006). The saturation of the apple linkage map with random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) markers was strongly improved within the last years (Liebhard et al. 2003a; Silfverberg-Dilworth et al. 2006). Time- and cost-efficient molecular techniques, as for example multiplex-polymerase chain reaction (PCR)-based methods (Frey et al. 2004), accelerated the development of genetic maps. Knowledge about the underlying genetics of resistance may facilitate the efficient breeding of pest-resistant apple cultivars. Molecular markers can be used to select apple cultivars based on their genome (MAS; Brown and Maloney 2003; Francia et al. 2005), even at the seedling stage (Mohan et al. 1997). Furthermore, MAS facilitates the combination of resistances to serious diseases and pests, and of high fruit quality (Fischer 1994; Mohan et al. 1997; Varshney et al. 2004), and it offers the possibility to pyramid two or more resistance genes promoting durable resistance (Mohan et al. 1997; Kellerhals et al. 2004). In the apple system, there was a focus on plant diseases, and QTL studies were carried out for resistance to apple scab [*Venturia inaequalis* (Cke.) Wint] (e.g. Liebhard et al. 2003b; Calenge et al. 2004), mildew [*Podosphaera leucotricha* Ellis and Everh.] (Calenge and Durel 2006), and fire blight [*Erwinia amylovora* (Burrill) Winslow et al.] (Calenge et al. 2005; Khan et al. 2006). Information on QTLs for tree growth and fruit quality traits is also available (Conner et al. 1998; King et al. 2000; Liebhard et al. 2003c; Segura et al. 2006). Recently, some basic information about markers associated to pest resistance was provided for three aphid species, namely the leaf-curling aphids (*Dysaphis* cf. *devecta* Wlk., species complex), the rosy apple aphids (*Dysaphis plantaginea* Pass.), and the woolly apple aphids (*Eriosoma lanigerum* Hausm.) (Roche et al. 1997; Bus et al. 2008; Stoeckli et al. 2008a). Evidence for a genetic basis of pest resistance in apple was given for several other herbivore species, for example for the brown-headed leafroller (*Ctenopseustis obliquana* Wlk.) (Wearing et al. 2003), the European red mite (*Panonychus ulmi* Koch) (Goonewardene et al. 1982), but information on the genetic basis of arthropod resistance in apple is still scarce. No gene region for *A. schlechtendali* resistance is known so far, and the present study is the first report of a QTL analysis for *A. schlechtendali* resistance in apple.

The aim of this study was to assess the genetic basis of resistance in apple to *A. schlechtendali*. Apple trees representing 160 different progeny genotypes were surveyed for rust mite infestation at each of three different study sites. Based on rust mite infestation, a QTL analysis was carried out using linkage map data available for a segregating F_1-cross of the apple cultivars 'Fiesta' × 'Discovery' (Liebhard et al. 2003a). Host-plant resistance to *A. schlechtendali* has been reported based on phenotypic evidence for 'Cox's Orange Pippin', the mother cultivar of 'Fiesta' (Easterbrook and Palmer 1996), which highlights the potential to identify QTLs for *A. schlechtendali* resistance in the 'Cox's Orange Pippin' pedigree. Effects of environmental variability on *A. schlechtendali* infestation, which may impede the detection of the genetic basis of resistance, were assessed by considering (1) climatic conditions, (2) the relationship of *A. schlechtendali* to other herbivores, and (3) the spatial variability of *A. schlechtendali* infestation at the different sites.

Materials and methods

Orchard characteristics and plant material

Apple rust mite (*A. schlechtendali*) abundance on apple trees was surveyed in Switzerland at the sites Zurich (Wadenswil; at 47°13′20″N, 8°40′05″E, 455 m altitude), Valais (Conthey; at 46°12′30″N, 7°18′15″E, 478 m altitude), and Ticino (Cadenazzo; at 46°09′35″N, 8°56′00″E, 203 m altitude), during two consecutive years, 2005 and 2006 (=Year 1 and Year 2). Climate data from March to August during the two consecutive years, and standard climate values (1960–1990) were retrieved from MeteoSwiss (http://www.meteoschweiz.ch). Highest mean temperatures (March to August) were measured at the Ticino site (16.8°C), followed by the Valais (15.2°C), and the Zurich site (13.8°C; Table S1). At the Valais site, the measured sum of rainfall (March to August; 300–400 mm) was half of the amount at the other sites (700–800 mm). Temperature was 1–2°C higher conferred to

standard temperature values, and at the Ticino site the measured sum of rainfall was 60–70% of the standard value (Table S1).

The studied apple trees represent F_1 progeny plants of the cultivars 'Fiesta' × 'Discovery' (*Malus* × *domestica* Borkh.). They were bud-grafted on M27 rootstocks in summer 1998 and planted in winter 1998/1999 at the three sites (Liebhard et al. 2003b). Tree-to-tree distance was 0.5 m (Zurich and Valais) and 1.25 m (Ticino), respectively. Rows were planted 3.5 m apart. The maximum number of genotypes present at all three sites was 160, and was lower for some analysis, as some trees died since plantation establishment. Orchards were treated with fertilizers and herbicides, but no insecticides, fungicides, and acaricides were applied.

Assessment of mites and other herbivores

In Year 1, the abundance of *A. schlechtendali* on each studied apple tree was quantified by (a) counting the number of all rusty leaves per tree and by (b) evaluating the number of mites per leaf extracted by filtration from a sample of 24 leaves per tree. In Year 2, the filtration method was applied only as the results of both methods were comparable. For the filtration method, the tree was divided into eight sectors (north, south, west, east; each bottom and top) and three leaves were sampled randomly from each sector to obtain a measure of mite abundance representing the whole tree and not only a tree part (Stoeckli et al. 2008b). Young leaves (the top three to five leaves of a shoot) were sampled as they generally show the highest *A. schlechtendali* infestation (Easterbrook and Palmer 1996). The leaves were suspended in 200 ml of a 0.1% Etalfix solution (surface-active agent; gvz-rossat, Otelfingen, Switzerland). After 5–7 h, the Etalfix solution was filtrated, using a multibranch filter system consisting of three filter holder support bases (Sartorius AG, Biotechnology Division, Dietikon, Switzerland), equipped with 'Biosart 250' funnels (250 ml, polypropylene material) and cellulose nitrate filters (diameter: 46 mm, pore size: 8 μm, white with black lines; Sartorius AG, Switzerland). *A. schlechtendali* on the filters were counted with a binocular. To assess a possible relationship between the number of mites per leaf and the leaf area (sum of the 24 collected leaves per tree), leaves from the Ticino site in Year 1 were exemplarily photographed with a digital camera (Nikon, Coolpix 990) together with a reference area of 1 cm^2, and total leaf area was determined using the software Adobe Photoshop CS2 for Mac OS X (following the method described in Mody and Linsenmair 2004).

To study the relationship between *A. schlechtendali* and different herbivore species, the abundance of three aphid and of two moth species was quantified for the same apple trees that were considered for rust mite assessments. The number of rosy apple aphid (*Dysaphis plantaginea* Pass.) colonies, the number of red-curled leaves caused by leaf-curling aphids (*D.* cf. *devecta* Wlk., species complex), and the number of green apple aphids (*Aphis pomi* De Geer), were counted three to four times from May to July at the three sites in the two consecutive years. The number of codling moth (*Cydia pomonella* L.) larval penetrations and the number of mines caused by the apple leaf miner (*Lyonetia clerkella* L.) were assessed in July and August (*C. pomonella*) and July (*L. clerkella*), at the Ticino and Valais site (*C. pomonella*) and at all three sites (*L. clerkella*), in Year 1 (*L. clerkella*) or the two consecutive years (*C. pomonella*).

QTL analysis

Abundance data of *A. schlechtendali* infestation were not normally distributed and a $\log_{10}(x+1)$ transformation was applied to normalize error distribution. QTL analyses of the number of rusty leaves per tree and the number of mites per leaf were carried out separately for each site and year using the software MapQTL® 4.0 (van Ooijen et al. 2002). The genetic linkage maps for both 'Fiesta' and 'Discovery' (single parent maps), used in QTL analysis, were already published (Liebhard et al. 2003a). Kruskal–Wallis tests and interval mapping (IM) were used for QTL analysis. Logarithm of odds (LOD) threshold values were determined by 1,000-fold permutation tests (MapQTL® 4.0) at a significance level of 95% (genome-wide; King et al. 2000). The 2-LOD support interval was calculated to estimate the position of significant QTLs with 95% confidence (King et al. 2000). Possible QTL interactions were tested by multiple QTL mapping (MQM) for QTLs with LOD scores exceeding the LOD threshold values in IM. The proportion of variation in mite infestation that can be explained by the genetic variation among the apple progenies was analyzed by broad-sense heritability, which was estimated by the formula $H^2 = \sigma_g^2/\sigma_p^2$ and $\sigma_p^2 = ([\sigma_g^2 + \sigma_e^2]/n)$, where σ_g^2 is the genetic variance, σ_p^2 is the phenotypic variance, σ_e^2 is the environmental variance and n is the number of replicates per genotype (Lauter and Doebley 2002). The outcome of the QTL analysis was confirmed by comparing *A. schlechtendali* abundance on apple genotypes amplifying the marker closest positioned to a QTL and genotypes not amplifying the specific markers (Mann–Whitney *U*-test). The SSR marker Hi03a10 (Silfverberg-Dilworth et al. 2006) was used to carry out a pedigree analysis of the identified QTL for rust mite resistance on the 'Fiesta' linkage group 7. PCR amplifications were performed in a 10 μl volume containing 5 μl of a DNA solution (1 ng/μl), 1× reaction buffer (Amersham Pharmacia, Dübendorf, Switzerland), 0.1 mM of each dNTP, 0.2 μM of dye-

labeled forward primer and 0.2 μM of reverse primer, and 0.7 U of Taq Polymerase (Amersham Pharmacia, Dübendorf, Switzerland) per reaction. PCRs were performed in a Gene Amp PCR system 9600 (Perkin Elmer, Foster City, CA), and microsatellite fragment lengths were scored with Genotyper 3.6 (Applied Biosystems).

Data analysis

Effect of genotype, site and year on *A. schlechtendali* infestation was assessed by a three factor mixed model ANOVA (number of mites per leaf), with year as within-subject effect, and genotype and site as between-subjects fixed effects. A one-way ANOVA was carried out to analyze the number of rusty leaves per tree, with genotype and site as fixed factors. Spearman's rank tests were applied to assess the relationship of (1) *A. schlechtendali* abundance between different sites and years, and of (2) *A. schlechtendali* number on the two neighbor trees (sum) on mite number on the specific individual trees. Only those trees were included in this analysis that had direct neighbor trees (a dead or missing tree was not regarded as neighbor tree). When multiple correlation tests were carried out the Benjamini–Hochberg procedure was applied to correct for false discovery rates (type I errors; Verhoeven et al. 2005). The distribution of *A. schlechtendali* on individual apple trees was analyzed by the index of dispersion (I_D; Southwood and Henderson 2000) using BiodiversityPro 1997 (Neil McAleece, P.J.D. Lambshead and G.L.J. Paterson; The Natural History Museum, London). I_D values significantly greater than the χ^2 statistic (0.025 probability level) indicate an aggregated distribution of the studied species (Ludwig and Reynolds 1988) in our study of *A. schlechtendali* on individual trees (some trees were strongly infested, whereas other trees were not infested at all). Potential effects of the spatial position of trees in the study sites on *A. schlechtendali* infestation were inferred from analyses of spatial autocorrelation, computing Moran's *I* (Legendre and Legendre 1998) and corresponding *z* values (significance levels) using the software CrimeStat III (Levine 2007). Values of *I* greater than the expected *I* indicate clustering while values of *I* less than the expected *I* indicate dispersion. Potential spatial patterns of *A. schlechtendali* infestation within the orchard were visualized by fitting trend surfaces on contour plots by kriging (best unbiased generalized least squares estimation) with an exponential covariance function (Venables and Ripley 2002). All statistical analyses were performed with SPSS 16.0 for Mac OS X (SPSS, Inc., Chicago, IL) and R 2.6.0 (R Development Core Team, Vienna).

Results

Evaluation of rust mite abundance

Highest numbers of apple rust mite (*A. schlechtendali*) per leaf were found at the Zurich site in Year 2 (mean: 6.2; Table 1), whereas lower infestation occurred at the Zurich site in Year 1 (mean: 0.6), at the Valais site (mean; both years: 0.6), and at the Ticino site (mean; Year 1: 1.4, Year 2: 0.3). The number of rusty leaves, caused by *A. schlechtendali* infestation in Year 1, was highest at the Zurich site (mean: 8.5; Table 1), compared to 5.6 and 5.4 at the Valais and Ticino site, respectively (Table 1). Maximal values showed that some highly infested trees occurred at the Ticino site (maximum number of mites per leaf: 84; Table 1). The number of mites per leaf and the number of rusty leaves per tree was significantly positively correlated (Spearman's rank test; Ticino, n=143, r_s=0.817, P<0.0001; Valais, n=142, r_s=0.902, P<0.0001; Zurich, n=153, r_s=0.585, P<0.0001). At the Ticino and the Valais site, the number of *A. schlechtendali* per leaf of the same tree genotype was significantly correlated in the two consecutive years (Ticino, n=143, r_s=0.232, P=0.005; Valais, n=142, r_s=0.180, P=0.003), but no significant correlation was detected at the Zurich site (n=153, r_s=0.123, P=0.131). For the same tree genotype, the number of *A. schlechtendali* per leaf and the

Table 1 *Aculus schlechtendali* infestation of progeny plants of the cross 'Fiesta' × 'Discovery' at three sites in two consecutive years

	Ticino			Valais			Zurich		
	n	Mean ± SE (Max)	*I*	*n*	Mean ± SE (Max)	*I*	*n*	Mean ± SE (Max)	*I*
No. mites per leaf									
Year 1	143	1.4±0.6 (84)	55	142	0.6±0.1 (6)	38	153	0.6±0.1 (13)	61
Year 2	143	0.3±0.1 (16)	19	142	0.6±0.2 (15)	26	153	6.2±0.8 (47)	73
No. rusty leaves									
Year 1	149	5.4±1.1 (83)	41	148	5.6±0.8 (51)	45	153	8.5±0.9 (54)	67

Number of studied genotypes (*n*), mean infestation±standard error (SE), maximum value (Max) and incidence (*I*=% infested trees) are presented. Mean and maximal values refer to the number of mites per leaf (extraction of 24 leaves per tree) in August in Year 1 and Year 2, and to the number of rusty leaves per tree in August in Year 1

Table 2 Comparison of *Aculus schlechtendali* infestation on individual apple genotypes at three sites assessed by Spearman's rank tests

	Correlation Ticino–Valais		Correlation Ticino–Zurich		Correlation Valais–Zurich	
	r_s	P	r_s	P	r_s	P
No. mites per leaf						
Year 1	0.315	**<0.0001**	0.026	0.758	0.259	**0.002**
Year 2	0.079	0.374	−0.003	0.975	−0.05	0.558
n	129		138		137	
No. rusty leaves						
Year 1	0.395	**<0.0001**	−0.082	0.326	0.359	**<0.0001**
n	140		144		143	

Significant correlations after FDR correction are in bold

number of rusty leaves were highly correlated in Year 1 among the Ticino and Valais sites, as well as among the Valais and Zurich sites, but not amongst the Ticino and Zurich sites (Table 2). No relation in the number of *A. schlechtendali* per leaf among the sites was detected in Year 2 (Table 2). The number of mites per leaf and the leaf area of the 24 assessed leaves (sum) were not significantly correlated at the Ticino site in Year 1 (Spearman's rank test; n=141, r_s=−0.156, P=0.065).

A. schlechtendali infestation was significantly influenced by apple genotype and site (ANOVA; Table 3). Year, as a within-subject effect, was not significant for the number of mites per leaf, but there was a significant year×genotype and year×site interaction (Table 3). Broad-sense heritability (H^2) for *A. schlechtendali* infestation was 19.9% (number of mites per leaf), and 46.2% (number of rusty leaves per tree, Table 4).

Table 3 Effect of genotype, site and year on *Aculus schlechtendali* infestation

	df	Mean square	*F* value	*P* value
No. mites per leaf				
Within-subjects effects				
Year	1	0.210	0.463	0.497
Year × genotype	157	0.548	1.205	<0.0001
Year × site	2	24.665	54.252	<0.0001
Error	278	0.455		
Between-subjects effects				
Genotype	157	0.682	1.249	0.050
Site	2	34.661	63.436	<0.0001
Error	278	0.546		
No. rusty leaves				
Genotype	157	0.417	1.865	<0.0001
Site	2	3.896	17.418	<0.0001
Error	290	0.224		

Evaluation of the number of mites per leaf assessed by a mixed model ANOVA and the number of rusty leaves per tree by one-way ANOVA

QTLs for rust mite resistance

Two significant QTLs were identified for *A. schlechtendali* resistance in apple on the 'Fiesta' linkage group 7 at the Zurich site in Year 1 (Fig. 1, Table 5). MQM mapping (multiple QTL mapping; data not shown) did not reveal any multiple linked QTLs and results were therefore based on IM. The closest markers to these QTLs were the AFLP marker E35M42-0146 at 20.2 cM and the RAPD marker AE10-400 at 45.8 cM (Table 5). The significant LOD scores at the marker positions were 4.3 (E35M42-0146) and 3.1 (AE10-400) for the number of mites per leaf (Zurich Year 1). For the number of rusty leaves per tree, the significant LOD scores at the marker positions were 6.0 (E35M42-0146) and 4.7 (AE10-400) (Zurich). The phenotypic variation explained by these markers ranged between 11–12% (number of mites per leaf) and 16–17% (number of rusty leaves per tree) for significant LOD scores (Table 5). The 95% confidence interval (2-LOD support interval) ranged from map position 15–25 cM (E35M42-0146) and 28–47 cM (AE10-400) (Fig. 1, Zurich Year 1). A lower *A. schlechtendali* infestation was found for apple genotypes amplifying the AFLP marker E35M42-0146 or the RAPD marker AE10-400 compared to apple genotypes not amplifying one of the markers in 14 of 18 surveys (Table 5). In the case of a significant QTL (Zurich), the difference was significant (Mann–Whitney, P<0.05; Table 5). A combined analysis of the AFLP marker E35M42-0146 or the RAPD marker AE10-400 did not reveal an interaction between the two markers. A lower *A. schlechtendali* infestation of the

Table 4 Broad-sense heritability (H^2) for *Aculus schlechtendali* infestation

Variance components	σ_g^2	σ_p^2	H^2
No. mites per leaf	0.045	0.227	0.199
No. rusty leaves	0.064	0.139	0.462

Calculation of genotypic variance (σ_g^2) and phenotypic variance (σ_p^2) based on mean square ANOVA results (cf. Table 3)

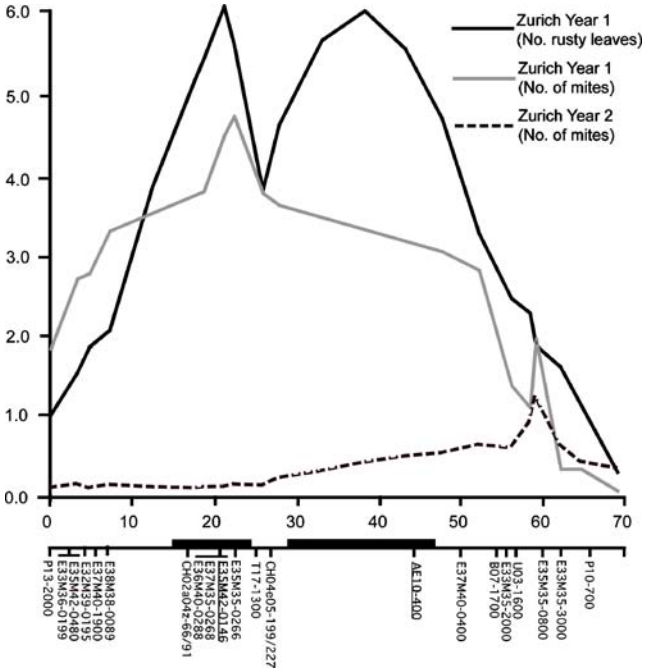

Fig. 1 QTLs for resistance of apple to *A. schlechtendali* identified on linkage group 7 of 'Fiesta' based on IM results. The *x* axis indicates the linkage map of 'Fiesta' in cM and the marker names; the *y* axis shows the LOD scores. The *solid black bar* indicates the 2-LOD support interval for the position of the QTL (Zurich Year 1). $\text{Log}_{10}(x+1)$ transformed data were used for QTL analysis. The markers closest positioned to the QTLs are *underlined*. LOD threshold levels at the Zurich site for the number of mites per leaf were 2.6 (Year 1) and 4.5 (Year 2), and for the number of rusty leaves the threshold level was 3.2 (Year 1)

'Fiesta' × 'Discovery' progeny amplifying the AFLP marker E35M42-0146 was found independently of the presence/absence of the RAPD marker AE10-400 (Table 6; Zurich Year 1). Similarly, *A. schlechtendali* infestation was lower or equal on genotypes amplifying AE10-400 compared to trees not amplifying AE10-400, independent of the presence/absence of E35M42-0146. The effect of the AFLP marker E35M42-0146 was stronger than the effect of the RAPD marker AE10-400 as differences between presence/absence of this marker are higher compared to the latter (Table 6). The origin of the QTL associated with *A. schlechtendali* resistance (AFLP marker E35M42-0146 at 20.2 cM) was traced back in the 'Fiesta' pedigree. The allele '240 bp' of the SSR marker Hi03a10 (Silfverberg-Dilworth et al. 2006), which is closely located and in coupling to the marker E35M42-0146 (5.8 cM distance between Hi03a10 and E35M42-0146), was found to be inherited from the cultivar 'Idared' and finally 'Wagener' (Fig. 2).

We additionally identified QTLs that were significant for one survey method, at one site, and in one of the two consecutive years (data not shown). The ALFP marker E35M41-0148 at 63.8 cM of linkage group 5 of 'Discovery' was closest positioned to a QTL that was significant for the number of mites per leaf at the Valais site in Year 2. The QTL had a LOD score of 3.1 and explained 9.5% of the phenotypic variability. Also for 'Discovery', the RAPD marker C05-1000 on linkage group 4 at 35.8 cM, and the allele '112 bp' of the SSR marker CH02g09 on linkage group 8 at 33.5 cM, were closest positioned to QTLs that were significant for the number of rusty leaves at the Zurich site in Year 1. The LOD scores of the QTLs were 3.2 and 2.0, and the phenotypic variability explained was 9.0% and 5.9%.

Spatial distribution of rust mites

The calculation of the index of dispersion (I_D) showed that *A. schlechtendali* infestation on individual trees was significantly aggregated ($P<0.0001$; Table S2). Some trees were strongly infested, whereas other trees were not infested at all, but there was no significant spatial pattern of *A. schlechtendali* infestation assessed by Moran's *I* (Table 7). This finding was in line with a visualization of the position of trees infested with *A. schlechtendali* (Fig. S1). No correlation between mite infestation on an individual tree and mite infestation on the two neighboring trees was found, neither for the number of rusty leaves nor for the number of mites per leaf (Table S3).

Relationship between rust mite abundance and co-occurring herbivore species

A significant positive relationship was found between the number of *A. schlechtendali* per leaf and the number of *A. pomi* per tree at the Ticino site in Year 1 (Spearman's rank test, Benjamini–Hochberg procedure; $n=143$, $r_s=0.284$, $P=0.001$), and at the Zurich site in Year 1 and Year 2 (Year 1, $n=153$, $r_s=0.222$, $P=0.006$; Year 2, $n=153$, $r_s=0.200$, $P=0.005$). A significant positive relationship between *A. schlechtendali* and *A. pomi* was additionally found for the number of rusty leaves per tree as measure for mite infestation in Year 1 (Ticino, $n=143$, $r_s=0.270$, $P=0.001$; Zurich, $n=153$, $r_s=0.204$, $P=0.011$). No significant relationship between infestation of the apple genotypes by *A. schlechtendali* and by the other surveyed herbivores *D. plantaginea*, *D.* cf. *devecta*, *C. pomonella*, and *L. clerkella* was identified ($P>0.05$ for all tests).

Discussion

The purpose of this study was to investigate the resistance of apple (*Malus* × *domestica*) to the apple rust mite (*A. schlechtendali*). Interactions between *A. schlechtendali* and other herbivores, climatic conditions at the study sites and

Table 5 QTLs identified for *Aculus schlechtendali* infestation in a segregating 'Fiesta' × 'Discovery' population on linkage group 7 of 'Fiesta'

Site and year		Locus	Closest marker[a]	LOD score (threshold)[b]	PVE[c]	Mean infestation (marker pres/abs)[d]
Number of mites per leaf						
		20.2	E35M42-0146 (+)			
Ticino	Year 1			0.8 (2.5)	2.9	0.7/1.0$^{n.s.}$
	Year 2			0.7 (2.4)	2.5	0.2/0.3$^{n.s.}$
Valais	Year 1			1.3 (3.0)	4.1	0.5/0.8*
	Year 2			0.3 (3.4)	0.7	0.8/0.4$^{n.s.}$
Zurich	Year 1			4.3 (2.6)	12.3	0.5/0.9*****
	Year 2			0.2 (4.5)	0.4	6.2/6.3$^{n.s.}$
		45.8	AE10-400 (−)			
Ticino	Year 1			0.8 (2.5)	7.9	1.2/0.8$^{n.s.}$
	Year 2			0.7 (2.4)	6.7	0.7/0.5$^{n.s.}$
Valais	Year 1			1.1 (3.0)	6.4	0.6/0.6$^{n.s.}$
	Year 2			1.2 (3.4)	9.1	1.3/0.4$^{n.s}$
Zurich	Year 1			3.1 (2.6)	11.0	0.4/0.8***
	Year 2			0.8 (4.5)	2.9	4.0/6.6$^{n.s.}$
Number of rusty leaves						
		20.2	E35M42-0146 (+)			
Ticino				0.7 (2.5)	2.2	4.6/6.0$^{n.s.}$
Valais				1.6 (3.4)	4.8	4.0/7.2****
Zurich				6.0 (3.2)	16.6	4.7/12.1*****
		45.8	AE10-400 (−)			
Ticino				0.8 (2.5)	6.0	5.0/6.8$^{n.s.}$
Valais				1.5 (3.4)	5.7	5.0/6.0*
Zurich				4.7 (3.2)	15.9	4.5/10.7****

QTL analysis was carried out for each year and site separately. Site and Year, genetic locus (locus in cM), closest marker, linkage phase, LOD score and threshold level at the locus of the closest marker, and phenotypic variance explained (PVE in %) based on IM are presented. Mean *A. schlechtendali* infestation for the two subpopulations of the 'Fiesta' x 'Discovery' progeny based upon the presence (pres) and absence (abs) of the nearest markers linked to the identified QTL on the 'Fiesta' chromosome. Significant LOD scores are highlighted

[a] Molecular marker closest to the likelihood peak of each QTL. Linkage phase information is provided as (+) or (−), indicating on which of the homologous chromosomes the marker is located

[b] LOD (logarithm of odds ratio) score and LOD threshold at the position of the closest marker. LOD threshold levels were derived by 1000-fold permutation tests (genome-wide)

[c] Phenotypic variance explained by the QTL

[d] Mean *A. schlechtendali* infestation for the two subpopulations of the 'Fiesta' x 'Discovery' progeny divided based upon the presence/absence of the nearest markers linked to a QTL on the 'Fiesta' chromosome. Different letters indicate significant differences between subpopulations (Mann–Whitney *U*-test). $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.005$; $^{****}P<0.001$; $^{*****}P<0.0001$. Sample size for the marker E35M42-0146 varied between 70–76 (pres) and 69–74 (abs). For the marker AE10-400 sample size varied between 46–52 (pres) and 53–56 (abs)

the spatial variability of *A. schlechtendali* infestation were assessed to elucidate QTL effects. We identified two significant QTLs associated with *A. schlechtendali* resistance on the 'Fiesta' linkage group 7. The AFLP marker E35M42-0146 at 20.2 cM and the RAPD marker AE10-400 at 45.8 cM were closest positioned to the QTLs. A significantly lower number of mites per leaf and a lower number of rusty leaves per tree were found for apple genotypes amplifying the AFLP marker E35M42-0146 compared to apple genotypes not amplifying the markers at the Zurich site in Year 1 and at the Valais site in Year 2. Referring to the second marker on the 'Fiesta' linkage group 7, the RAPD marker AE10-400, the linkage map at this region is not well saturated with molecular markers. QTL significance may disappear when including more markers in the QTL analysis. We did not find an interaction between the two markers and they seem to be linked, as 70% of the apple genotypes amplifying for E35M42-0146 additionally amplify the marker AE10-400. These findings may partly explain the QTL at the region of the RAPD marker AE10-400. Broad-sense heritability (H^2) amounted to 19.9% (number of mites per leaf), and 46.2% (number of rusty leaves), respectively. While some putative QTLs for resistance to *A. schlechtendali* were also found on the 'Discovery' linkage groups 4, 5, and 8, the linkage group 7 of 'Fiesta' appears to be strongly related to resistance against different pests and diseases. Besides the two newly identified QTLs associated to resistance to *A. schlechtendali*, a QTL for fire blight resistance (Calenge et al. 2005; Khan et al. 2006) and one for *D.* cf. *devecta* resistance

Table 6 Combined analysis of the two markers at the peak of the QTLs E35M42-0146 and AE10-400 ('Fiesta') that where significantly linked to *Aculus schlechtendali* resistance at the Zurich site in Year 1

		AE10-400	
		Presence	Absence
No. mites per leaf			
E35M42-0146	Presence	0.39±0.10	0.38±0.13
	Absence	0.63±0.18	0.92±0.11
No. rusty leaves			
E35M42-0146	Presence	0.39±0.07	0.51±0.15
	Absence	0.67±0.17	0.89±0.08

The phenotypic trait (number of mites per leaf and number of rusty leaves) was divided in four subpopulations based on the presence and absence of the markers, and average values ($\log_{10}(x+1)$—transformed) were analyzed

(Roche et al. 1997; Stoeckli et al. 2008a) were previously identified on this linkage group. The specific gene regions of these QTLs are probably not overlapping. The fire blight QTL is positioned on the bottom of the linkage group (46.5–51.5 cM), whereas the QTL for *D.* cf. *devecta* resistance was identified at its top (0–5 cM).

The genetic basis of apple resistance to *A. schlechtendali* is underlined by the finding that rust mite infestation of individual trees representing unique genotypes was highly aggregated (I_D, Table S2), and that genotype was a significant factor explaining the number of rusty leaves per tree (ANOVA; Table 3). Furthermore, we detected a significant correlation of *A. schlechtendali* infestation of individual apple genotypes between the sites Ticino and Valais, as well as between the sites Valais and Zurich in Year 1. In combination with the identified QTLs, these phenotypic findings provide further evidence for the genetic basis of rust mite resistance in apple, which is supported by results from other studies showing that rust mite infestation may vary on different apple cultivars (e.g. Graf et al. 1998; Spieser et al. 1998; Duso et al. 2003).

A number of apple cultivars described as resistant to *A. schlechtendali*, such as 'Cox's Orange Pippin' (Easterbrook and Palmer 1996), 'Florina' (Graf et al. 1998), 'Glockenapfel' (Höhn and Höpli 1990), 'Golden Delicious' (Herbert 1974; Spieser et al. 1998), 'Jonagold' (Höhn and Höpli 1990), 'McInosh' (Downing and Moilliet 1967), 'N.Y. 18491' (Duso et al. 2003) or 'Red Delicious' (Downing and Moilliet 1967; Table S4), are highly promising to be tested for the SSR marker allele associated with the described QTL (allele '240 bp' of Hi03a10 on 'Fiesta' linkage group 7). However, the pedigree analysis of 'Fiesta' revealed that the SSR marker allele was inherited from the cultivar 'Wagener' to 'Idared', but was not present in 'Cox's Orange Pippin'. Host-plant resistance to *A. schlechtendali* has not yet been reported for the cultivars 'Wagener' and 'Idared', and phenotypic field surveys are therefore highly encouraged. The phenotypically observed resistance of 'Cox's Orange Pippin' (Easterbrook and Palmer 1996) may be based on genetic factors, or its expression may be strongly affected by environmental conditions.

The identified QTL for the number of mites per leaf was significant for the Zurich site in Year 1 and the Valais site in Year 2, but it was not significant for the second year and the Ticino site. This observation may reflect environmental variability, which may partly explain different infestation patterns and the instability of QTLs among sites (Walde et al. 1997). The potential influence of environmental variability on rust mite infestation is supported by the finding of a lacking correlation of *A. schlechtendali* infestation of individual apple genotypes amongst the Ticino and Zurich sites in Year 1. In general, all three sites differed markedly in climate conditions. Temperature at the Ticino and the Valais site was higher compared to the Zurich site, and field observations revealed that tree phenology in the Zurich orchard was approximately 2 weeks delayed compared to the Ticino site. Besides climate, other environmental factors such as the composition of the orchard fauna may affect mite distribution and mask the effects of host tree genotype. Host-tree infestation by aphids, for example, affects leaf growth and, thus, probably changes habitat and resources for rust mites. There was a significant positive correlation between infestation by *A. schlechtendali* and the aphid *A. pomi* at the Ticino site in Year 1, and at the Zurich site in Year 1 and Year 2. *A. pomi* infestation was higher at the

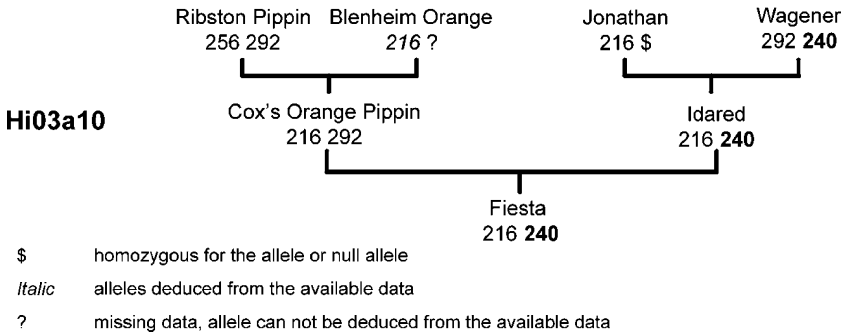

Fig. 2 Analysis of the pedigree of the apple variety 'Fiesta' with the SSR marker associated with one of the QTLs for resistance to *A. schlechtendali* (AFLP marker E35M42-0164). The SSR marker allele associated with resistance is in *bold* (allele '240 bp' for Hi03a10)

Table 7 Spatial distribution pattern of *Aculus schlechtendali* infestation at the Ticino, Valais and Zurich site in two consecutive years (mean values of different surveys within a year) assessed by Moran's *I*. Values of *I* greater than a randomly expected *I* indicate clustering while smaller values of *I* indicate dispersion

	Moran's I	I randomly expected (SD)	Normality significance (z)	Randomization significance (z)
No. rusty leaves				
Ticino	−0.012	−0.007 (0.012)	−0.398	−0.412
Valais	0.009	−0.007 (0.022)	0.704	0.713
Zurich	0.032	−0.007 (0.019)	1.955	**1.981**
No. mites per leaf				
Ticino				
Year 1	−0.004	−0.007 (0.013)	0.262	0.375
Year 2	−0.011	−0.007 (0.013)	−0.338	−0.409
Valais				
Year 1	0.005	−0.007 (0.024)	0.523	0.532
Year 2	−0.022	−0.007 (0.024)	−0.619	−0.676
Zurich				
Year 1	0.014	−0.007 (0.019)	1.100	1.211
Year 2	0.001	−0.007 (0.019)	0.400	0.405

The *z* values were compared to a standard normal table, and absolute values greater than 1.96 indicate a spatial autocorrelation at a 5% significance level. Significant *z* value are in bold. Sample size was $n=143$ for the Ticino, $n=142$ for the Valais, and $n=153$ for the Zurich site

Ticino and Valais sites than at the Zurich site and may have contributed to instable QTL effects considering sites. Although high herbivore infestation of an individual tree may serve as a source of infestation by the same herbivore of the neighbor trees (neighborhood effect), there was no correlation between mite abundance on an individual apple tree and mite abundance on the two neighbor trees. Similarly, there was no significant effect of the spatial position of trees in the study plots on distribution of *A. schlechtendali*. Therefore, neighborhood effects or spatial autocorrelation can be ruled out as explanation for the QTLs in different environments and years.

The apparent influence of environmental conditions on the variable and partly weak expression of the described QTLs has to be taken into account when considering these QTLs for breeding programs or orchard management decisions. The stability of the QTLs in different genetic backgrounds and under different environmental conditions should be evaluated and further molecular markers should be developed to saturate the described QTL regions. Consideration of SSR markers that are closely positioned to the QTLs will enhance the reliability and efficiency of marker assisted selection (MAS; Mohan et al. 1997; Francia et al. 2005). Besides a focus on the genetic basis of *A. schlechtendali* resistance in apple, knowledge about biotic and abiotic factors related to mite resistance will facilitate rust mite control in apple orchards, requiring field evaluations to receive environment-specific information. The complexity of breeding pest-resistant cultivars is not only due to the mentioned environmental factors but also to the usually quantitative genetic background of arthropod resistance. Nonetheless, SSR marker alleles such as allele '240 bp' of the SSR marker Hi03a10 that are closely positioned and in coupling to the identified QTLs may be used as a starting point to screen existing apple cultivars for resistance to *A. schlechtendali* and to identify resistant parents that can be used in MAS to develop new resistant apple cultivars.

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