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to ash dieback “**

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1. Abstract

Common ash (*Fraxinus excelsior*) has recently become seriously endangered throughout Europe by a fungal disease (ash dieback) that is caused by the ascomycete *Hymenoscyphus fraxineus*. This disease developed into a threat for forest management as it leads to high mortality of ash trees and it raised concerns for the future of common ash. Previous studies have revealed differences in susceptibility among ash individuals providing evidence of genetically based resistance or tolerance against the pathogen.

The aim of the study was to identify if a correlation between damage intensity of mature trees and their offspring exists that indicates inherited resistance. Crown damage from natural infection on trees and shoot damage from saplings were assessed in two isolated ash stands in Austria. A range between nearly healthy to severely damaged individuals were found on both sites, with many moderately damaged and no dead trees. Parentage analysis, carried out with molecular markers, resulted in 26 offspring that could assigned to both parents, and 58 that could assigned to one parent. A total of 45% of the offspring were left unassigned.

No significant correlation of damage intensity between parent and offspring could be verified using Spearman's rank correlation analysis (offspring with both parents: $r_s = 0.098$, $P = 0.318$; offspring with one parent: $r_s = -0.082$, $P = 0.272$), suggesting a lack of evidence of truly resistant or tolerant trees at the study sites. Supported by results of other studies, it is considered that resistant or tolerant individuals within European ash populations occur in low frequency and are probably better detectable when populations are severely damaged. As most of the previous studies about estimations of inheritance of possible resistance were carried out in controlled environments like progeny trials or seed orchards, results from the current investigation suggests a more complex mechanism of susceptibility differences under natural heterogeneous conditions.

The robustness of the observed resistance in previous studies should to be evaluated. Further analyses are needed to obtain a better understanding of gene-

environment interactions and individual infection pressure against ash dieback in natural environments. Large-scale dieback of *Fraxinus excelsior* trees is becoming a realistic scenario for the near future, and therefore identifying and propagating non-susceptible ash trees is an important challenge.

2. Zusammenfassung

Die Gemeine Esche (*Fraxinus excelsior*) ist in ganz Europa durch eine invasive Pilzkrankheit (Eschentriebsterben) ernsthaft gefährdet. Verursacht wird die Krankheit durch den Schlauchpilz *Hymenoscyphus fraxineus*. Beachtliche Schäden und die hohe Mortalität von Eschen führen zu großen Problemen für die Forstwirtschaft und machen die Zukunft dieser Baumart ungewiss. Studien haben hohe Unterschiede in der Befallsintensität zwischen Individuen gezeigt. Vermutet wird heute, dass diese Unterschiede genetische Resistenz oder Toleranz einzelner Bäume gegen die Krankheit darstellen und möglicherweise weitervererbt werden.

Das Ziel der Studie war es, einen Zusammenhang zwischen der Befallsintensität von Bäumen und deren Nachkommen herzustellen, um eine mögliche, vererbte Resistenz aufzudecken. In zwei relativ isolierten Eschenbeständen in Österreich wurden der Grad der Kronenschädigung an Bäumen und der Grad der Schädigung an Jungtrieben gemessen. Beide Standorte zeigten eine Bandbreite zwischen nahezu gesunden und stark geschädigten Individuen, die meisten waren mäßig geschädigt. Tote Bäume wurden nicht gefunden. Elternschaftsanalysen wurden mittels molekularen Markern durchgeführt. 26 Jungbäumen konnten beide Eltern und 58 einem Elternteil zugeordnet werden. Für 45% der Jungbäume wurden keine Eltern gefunden.

Spearman's Rangkorrelation zeigte keinen signifikanten Zusammenhang der Befallsintensität zwischen Eltern und Nachkommen (Jungbäume mit beiden Eltern: $r_s = 0.098$, $P = 0.318$; Jungbäume mit einem Elternteil: $r_s = -0.082$, $P = 0.272$). Das könnte auf ein Fehlen resistenter oder toleranter Bäume in beiden Standorten hinweisen. Bekräftigt von den Ergebnissen anderer Studien, ist davon auszugehen, dass resistente oder tolerante Individuen innerhalb europäischer

Eschenpopulationen in eher geringer Häufigkeit auftreten und wahrscheinlich besser erkennbar sind, wenn die Population sonst stark geschädigt ist.

Da die meisten Studien, in mehr oder weniger kontrollierter Umgebung von Samenplantagen durchgeführt wurden, könnte diese Studie die Komplexität der Ursachen für unterschiedliche Befallsintensitäten unter natürlichen, heterogenen Bedingungen aufzeigen. Die Robustheit beobachteter Resistenzen in diesen Studien müsste weiter evaluiert werden. Weitere Analysen sind erforderlich um ein besseres Verständnis über Gen-Umwelt Interaktionen und individuellen Befallsdruck gegen das Eschentriebsterben in natürlicher Umgebung zu bekommen. Massive Dezimierung von *Fraxinus excelsior* Populationen in Europa wird als Szenario immer wahrscheinlicher. Es ist daher zu empfehlen, nahezu gesunde Bäume europaweit zu identifizieren und ein geeignetes Management Programm zu entwickeln.

3. Keywords: ash dieback, common ash, disease tolerance, *Hymenoscyphus fraxineus*, parentage analysis, resistance

4. Introduction

Common ash (*Fraxinus excelsior*) is a widely distributed tree species throughout temperate Europe and of substantial ecologic and economic importance. The species tolerates a wide range of environmental conditions from riparian to mountain habitats (Pautasso et al. 2013). Compared to beech, common ash is adapted to sites that are moister or drier and it prefers nutrient rich soils (Marigo et al. 2000, Thomas 2016). Although the seedlings are shade tolerant, good light conditions are needed to compete with other broad-leaved tree species (Marigo et al. 2000). Common ash has a special mating system with male, female and hermaphrodite individuals and its pollen and seeds (samaras) are wind-dispersed (Wallander 2008, Morand-Prieur et al. 2003).

Recently, common ash has become highly threatened by a fungal disease (ash dieback) caused by the ascomycete *Hymenoscyphus fraxineus* (Gross et al. 2014), previously known as *H. pseudoalbidus*, with its anamorph stage *Chalara fraxinea* (Kowalski 2006, Queloz et al. 2011). The pathogen was most likely introduced from East Asia (Zhao et al. 2012, Gross et al. 2014) and was first observed in Poland in the early 1990s (Przybyl 2002, Pautasso et al. 2013). Since then, the pathogen spread all over Europe. First ash dieback symptoms were observed in Austria in 2005 at a few sites in Lower Austria, Upper Austria and Styria and subsequently spread to all federal provinces of Austria by 2009 (Cech 2006a, b; Cech et al. 2012).

The symptoms of ash dieback range from necrotic lesions and wilting on ash leaves and petioles to necrotic lesions on branches, shoots and stem, leading to wood discoloration and crown dieback and in most severe cases to death of the tree (Cech 2006b, Bakys et al. 2009). Through wind dispersal, the fungus infects ash leaves during summer, growing into shoots and twigs and overwinters on infected leaf stalks in the litter after leaf fall (Kräutler & Kirisits 2012, Landolt et al. 2016). Ash dieback attacks all tree age classes, although symptoms progress more severely and more rapidly in younger individuals, causing immense problems for rejuvenation (Heinze et al. submitted, Keßler et al. 2012). As the pathogen causes

high mortality of ash trees, ash dieback has become a serious problem raising concerns about the future of the species.

In many European countries, studies in seed orchards, progeny trials and also in natural stands during the last years aimed for a better knowledge of the pathogen and its impact on Common ash. Although there is an overall progression of the disease, several studies have revealed differences in symptom intensity among individuals providing the evidence of genetically based variation in susceptibility suggesting resistance or tolerance (Pliura et al. 2011, McKinney et al. 2011, Stener 2012, Kjaer et al. 2012). Results from inoculation studies show that the pathogen was less penetrative in less susceptible individuals suggesting a better response mechanism of the host (McKinney et al. 2012, Cleary et al. 2014). Possible resistance against ash dieback was previously estimated based on covariance and estimated heritability among half-sibs and full-sibs in progeny trials suggesting that parents that are less susceptible transmit the resistance to their offspring (Kjaer et al. 2012; Lobo et al. 2014; Lobo et al. 2015).

The aim of this study was to investigate how closely symptom intensity of saplings and their parents are correlated, investigated in natural ash stands by direct parent offspring comparisons. Positive correlations should therefore imply a likely inheritance of resistance to ash dieback.

5. Materials and Methods

5.1. Study sites and sampling

Preceding requirements were defined for selecting suitable sampling sites. The optimal site must represent a fairly isolated ash stand with enough rejuvenation and healthy to severely damaged mature trees. Two study sites were finally chosen (figure 1).

The first sampling site is a small ash stand within the Johannser Kogel, which is a natural forest reserve of about 45 ha and is located in the northwestern part of the Lainzer Tiergarten with the geographical coordinates N 48° 11' and E 16° 12' (Türk & Pfleger 2008). The Lainzer Tiergarten is conservation reserve and a NATURA 2000 site of 2.460 ha with a high biodiversity, old trees and a high amount of deadwood located in the western part of Vienna and the eastern part of the biosphere reserve Wienerwald (<https://www.wien.gv.at/umwelt/wald/erholung/lainzertiergarten/lebensraum/lage.html>, 05-20-2016). The natural forest reserve Johannser Kogel has an altitudinal range of 290 to 377 m and an average annual precipitation of about 650 mm (Türk & Pfleger 2008). It is dominated by an oak-hornbeam forest with characteristic old oak trees which are up to 400 years old (Türk & Pfleger 2008). The ash stand has an area of about 2 ha and is found on the top of the hill in the middle of the reserve and is further described as a so called “hilltop ash forest” (“Gipfeleschenwald”) (Willner 1996). Hilltop ash forests ash-dominated forests that often form pure stands on hilltops and on northern slopes (Willner 1996). The hilltops may represent atypical sites where ash find better conditions and out-compete other forest trees (Willner 1996). The ash forest at Johannser Kogel is mixed with field maple (*Acer campestre*).

The second sampling site, Siegenfeld, is a small ash stand of about 1 ha area located in a forest between Siegenfeld and Heiligenkreutz (N 48° 03' and E 16° 9'). It is surrounded by a spruce forest in the north, west and south and confined by a forest street in the east. Other tree species found in the ash stand are beech, maple

and larch. The altitude is about 400 m and mean annual precipitation is about 688 mm (<http://de.climate-data.org/location/142025/>, 02-19--2016). In 2007, the Federal Research Centre for Forests (BFW) started a monitoring program on 50 plots in Lower Austria where crown dieback intensity was estimated on 20 trees per plot. Two years later, the Austrian Forest Inventory included 1200 more plots for ash dieback monitoring. Siegenfeld is one of those plots in Lower Austria (Cech et al. 2012). Most of the 20 trees from Siegenfeld showed less symptom intensity from 2007 till 2009 (Cech et al. 2012), which made Siegenfeld as one of the healthiest monitoring plots by now (personal information by Katharina Schwanda).

Leaf or cambium samples were collected at both sites in June, July and August 2015 from 160 saplings and all mature trees observed (a total of 134 trees). Ash dieback was recorded as the degree of crown dieback. Mature trees were classified into one of six damage classes (figure 1B). Class 1: trees with no or few symptoms and <10% loss of crown foliage; class 2: trees between 10% and 25% crown foliage; class 3: trees with more than 25% but less than 50% crown foliage; class 4: between 50% and 75 %; class 5: between 75% and < 100% crown foliage and trees that had died from infection were scored as Class 6 (100% crown defoliation). Saplings were categorized in same damage classes, but instead crown damage, shoot damage was recorded (figure 2B). Additionally the diameter of breast height (DBH) of each tree and the height of each sapling were measured. Saplings were only sampled when they exceeded the height of about 60 cm, so that symptom identification and damage class categorization were confident enough.

One leaf per sapling and either a leaf (caught by a slingshot) or a bark plug per mature tree was collected. The bark plug from which cambium tissue can be sliced, was cut out of the trunk with a 1 cm diameter leather punch. After sampling the material was immediately dried in silica gel and kept at room temperature prior to DNA extraction.

5.2. *DNA extraction*

On average, 25 – 45 mg dried leaf or cambium tissue per individual was put in 2 mL tubes together with two 3 cm diameter and one 4 cm diameter glass balls (for cambium tissue steel balls were used), one spatula tip of glass powder, activated charcoal, polyvinyl pyrrolidone (PVP 40,000) and sodium metabisulfite. For homogenization, the material was frozen in liquid nitrogen for two minutes and grounded with a TissueLyser (QIAGEN) at 25 Hz for two minutes. The process was repeated a second time. The DNA was extracted using the Invisorb® Spin Plant Mini Kit from STRATEC Molecular, applying the protocol recommended for the kit. But instead of Lysis Buffer P, provided by the kit, a mixture of 800 µL 2x CTAB Buffer (100 mM Tris-HCL pH 8.0, 1.4 M NaCL, 25 mM EDTA pH 8.0) plus 1.6 µL β-Mercaptoethanol and 1 µL proteinase K was used for lysis. Additionally 40 µL RNase A (10 mg/mL) were added to each sample before the binding process. To determine DNA concentrations a NanoDrop 1000 Spectrophotometer (Thermo Fischer Scientific) was used.

5.3. *DNA amplification and fragment analysis*

DNA concentrations for all samples were below 20 ng per µL. Therefore, DNA extractions were maintained undiluted for polymerase chain reaction. Nine nuclear microsatellite loci were used for amplification (Femsatl-4, Femsatl-10, Femsatl-11, Femsatl-12, Femsatl-16, Femsatl-19, M230, FR639485 and FR646655) (Lefort et al. 1999; Brachet et al. 1999; Beatty et al. 2015). Each forward primer was labelled with a fluorescent dye. PCRs were carried out using the QIAGEN Type It Microsatellite Kit. The amplification reactions were performed on a PTC-100® Thermal Cycler (BIO RAD) under the following conditions: An initial denaturation step of 5 min at 95°C, 28 cycles of denaturation at 95°C for 30s, annealing with corresponding temperatures according to table 1 for 90s and extension at 72°C for 30s and a final extension step at 60°C for 30 min.

A CEQ 8000 Beckman Coulter Sequencer was used to visualize the PCR products based on fragment length polymorphism, as compared to CEQ DNA Size

Standard Kit-400. Allele assessment, binning and calling were carried out using the fragment analysis tool of GenomeLab GeXP Beckman Coulter software, with additional visual inspection of peaks.

5.4. *Parentage Analysis*

CERVUS 3.0.7 software (Kalinowski et al. 2007; Marshall et al. 1998) was used for parentage analysis and for calculating other parameters, such as expected (H_e) and observed (H_o) heterozygosity, polymorphic information content (PIC), average non-exclusion probability for one candidate parent (NE-P1), average non-exclusion probability for a candidate parent pair (NE-PP), Hardy Weinberg equilibrium (HW) and estimated null allele frequency (NULL). All of these parameters give information about the loci and their suitability for parentage analysis.

For parentage assignment, the program uses likelihood, a well-established statistical method (Marshall et al. 1998). Parentage is assigned to a candidate parent if the likelihood ratio is large relative to the likelihood ratio of alternative candidate parents. The likelihood ratio is expressed as LOD scores (logarithm of the likelihood ratio) (Marshall et al. 1998). Candidate parents with negative LOD scores are less likely to be the true parent. If two or more parents have positive LOD scores, Marshall et al. (1998) defined the logarithm of the ratio of likelihood to be delta (difference in LOD scores). In a simulation of parentage appropriate LOD and delta scores for valid parentage assignment are generated. The advantage in the use of CERVUS lies in the allowance for mistyping and missing data for individuals at a specified number of loci.

Parentage analysis for one parent (implemented by the maternity analysis function of CERVUS) and parent-pair analysis were carried out for both sites separately. Prior to parentage analysis, a simulation of parentage was performed with 10,000 offspring, an error rate of 0.01 at strict (95%) and relaxed (80%) confidence levels. As additional parameters candidate parents were set to 100 for Johanner Kogel with a 0.75 proportion of candidate parents sampled and 60 candidate parents with a 0.90 proportion of candidate parents sampled for Siegenfeld. The proportion of sampled candidate parents were estimated by field observation,

as the occurrence of unsampled trees in the proximity of the stands cannot be excluded with certainty. Unsampled candidate parents were estimated to be at low frequency in Siegenfeld and at moderate frequency in Johannser Kogel.

5.5. *Statistical analysis*

To estimate the significance of correlation between damage class of parent and offspring, a one-tailed Spearman's rank correlation analysis was calculated using SPSS Statistics 23 software. The calculation was applied separately for those offspring where both parents were assigned (Spearman's rank correlation coefficient between the damage class of the offspring and the averaged mean damage class of both parents) and for those where only one parent was assigned (Spearman's rank correlation coefficient between the damage classes of the offspring and the one parent). The boxplot (DBH for damage class) was done in SPSS, whereas the tables and the bar diagram were done in Microsoft Excel 2013. Figure 1 was created with ArcMap 10.2 software from ESRI.

6. Results

6.1. *Damage assessment*

Both sites show a range of slightly to severely damaged individuals, but most mature trees had a crown damage intensity between 10 to 50% and were assessed to damage classes 2 and 3 (54% of the trees from Siegenfeld and 67% from Johannser Kogel). Percentages of assessment to damage class 1 were higher in Siegenfeld than in Johannser Kogel (figure 3). No tree was observed to have lost the whole crown foliage (damage class 6).

Six saplings in Siegenfeld had only wilted leaves or no leaves and severely damaged shoots, so they were classified as dead (figure 2B). In Siegenfeld, 23 saplings were classified into damage class 1, and in Johannser Kogel, 14 saplings. More saplings from Johannser Kogel were assessed to damage class 2 (22) than from Siegenfeld (12). In general, both sites were similar regarding to damage in-

tensity. Around 45 % of the individuals had less than 25% crown or shoot damage (damage class 1 and 2) and more than the half of the individuals showed severe damage intensity (damage class 3, 4, 5).

In Siegenfeld, the mean height of the saplings was 99.3 cm ranging from 70 to 170 cm, and in Johannser Kogel mean height was 96.5 cm with a range from 65 to 190 cm. The mean diameter of the mature trees at breast height (DBH) was 35 cm in Siegenfeld and 39.7 cm in Johannser Kogel. Johannser Kogel had some older trees with DBH diameters of around 60 cm.

6.2. *Allele frequency*

A total of 285 individuals from 294 sampled were genotyped for nine microsatellite loci. DNA from one ash tree and eight saplings (including all of the six dead saplings from Siegenfeld) couldn't be amplified, likely due to insufficient DNA quality. These individuals were not considered for further analysis.

The loci showed high allele numbers (N) and revealed that most of the loci are highly polymorphic (table 1). The highest allele variation was shown by locus Femsatl-10 with 48 alleles, whereas the lowest variation with seven alleles exhibited Femsatl-16. 183 alleles were detected in 161 individuals from Johannser Kogel with an average number of 20.3 alleles per locus and 166 alleles were detected for 124 individuals from Siegenfeld with an average number of 18.4 alleles per locus. The mean proportion of loci typed exceeded 0.99 for both sites. Mean expected heterozygosity (He) was 0.75 for Johannser Kogel and 0.80 for Siegenfeld. Observed heterozygosity (Ho) ranged from 0.38 to 0.85 for Johannser Kogel, and from 0.29 to 0.87 for Siegenfeld. Expected heterozygosity was higher than observed in most of the loci and in both sites. Mean polymorphic information content was 0.71 for Johannser Kogel and 0.77 for Siegenfeld. Combined non-exclusion probability for the first parent and for the parent pair were $5.70E-03$ and $6.00E-07$ for Johannser Kogel and $8.92E-04$ and $8.73E-09$ for Siegenfeld. Deviations from Hardy-Weinberg equilibrium were detected at five loci in Johannser Kogel and at one locus in Siegenfeld. The estimated frequency of null alleles ranged from -0.003

(FR639485, Johannser Kogel) to 0.5 (Femsatl-12, Siegenfeld) with a mean value of 0.072.

6.3. *Parentage assignment*

The assignment rate for the parent-pair analysis in Johannser Kogel was 7.5 % implying that five saplings from Johannser Kogel were assigned both parents at relaxed confidence level and one at strict confidence level. In Siegenfeld, the assignment rate for the parent-pair analysis was higher with 27.7%. Both parents were determined for 20 saplings from Siegenfeld at a relaxed confidence level and 10 at strict confidence level.

Maternity analysis resulted in a 48.7% assignment rate for Johannser Kogel (42 saplings, 20 at strict confidence level) and the assignment rate for maternity analysis for Siegenfeld exceeded 30% (all of the assignments are with strict confidence). For 41.6 percent of the saplings from Siegenfeld and 43.8 percent from Johannser Kogel, no parent was assigned.

6.4. *Statistical analysis*

Spearman's rank correlation coefficients showed no significant association between damage classes of offspring and parents, neither for parent-pair, nor for maternity analysis (table 2). In addition, a Wilcoxon–Mann–Whitney test was calculated between damage classes of parents and high/low damage of offspring (damage class one and two were put together and categorized as low damage whereas class three, four, five were set as high damage). The results showed no significant difference (results not shown).

The boxplot (figure 4) shows that thin, young trees tend to be more affected by ash dieback than thick, old ones.

7. Discussion

7.1. Genetic parameters and parentage assignment

The majority of the loci used in this study were highly polymorphic with an average of 19 alleles per locus. Expected heterozygosity was high with an average of 0.79. Similar observations were made for the same microsatellite loci in previous population genetic studies on *F. excelsior*, suggesting high genetic diversity within populations (Heuertz et al. 2001, Hebel et al. 2006, Beatty et al. 2015). Allelic diversity did not differ much between Johannser Kogel and Siegenfeld (Mean number per loci: 20.3 and 18.4 respectively). Both sites had nearly the same set of alleles with few private alleles at each site. Therefore, genetic differentiation between both sites is assumed to be low (Heuertz et al. 2001, Hebel et al. 2006, Ballian et al. 2008).

Surprisingly, positive assignments of at least one parent were made only for c. half of the saplings, and for only 17% of the saplings both parents could be assigned. Although it was tried to sample all putative parents in both fairly enclosed stands, likely trees that grow outside the sampled stands are these missing parents. Another possible explanation for the low assignment rate could be genotyping or binning errors which would probably lead to undetected parentage, although CERVUS uses likelihood equations that take into account of such errors.

High combined exclusion probability (99%) across all loci for unrelated parents suggests low error rate in parentage assignment and shows that the microsatellite set used was rather adequate. However, a few loci showed problematic values that would lead to issues in parentage analysis and therefore need some discussion. The estimated frequency of null alleles displayed high values at some loci (0.33 and 0.5 at Femsatl-12, 0.09 at Femsatl-10) (table 1). Null alleles consistently do not amplify during PCR and can thus be not detected, leading to false assessments of heterozygotes as homozygotes (Kalinowski 2006). They are problematic in parentage analysis and appear sometimes simultaneously with deviations from the Hardy-Weinberg equilibrium (Pemperton et al. 1995), which was

also the case for five loci in Johannser Kogel and at one locus in Siegenfeld. Estimation of null allele frequencies does not necessarily imply that a null allele is present. If no parent-offspring relationship is definitely known, it is difficult to identify a null allele with certainty (Dakin 2004). Although Femsatl-12 was problematic, because of high estimated null allele frequency values and deviation from the Hardy-Weinberg equilibrium, it was included in parentage analysis, because CERVUS tolerates null alleles at low or moderate frequency. A general observation is that this locus amplifies well in Western Europe, less well in Central Europe, and very poorly in Eastern Europe (Heinze and Fussi submitted, their Table 4), which points at a geographical clustering of null alleles.

Another more likely explanation for the low assignment rate is that most of the saplings had been sired by trees that grow outside of the study stands suggesting high seed and pollen inflow from the surroundings. Similar results have been reported by Lobo et al. (2015), where paternity analysis in two Danish seed orchards resulted in an assignment rate of less than 40%. They suggested the small size of the seed orchard as one possible explanation (12 putative parents in the orchard). Analysis of seed dispersal in isolated, semi-continuous ash populations in Ireland carried out by Beatty et al. (2015) indicates the potential for seed dispersal over hundreds of meters. Bacles et al. (2005) and Bacles and Ennos (2008) estimated pollen dispersal in fragmented ash populations in Scotland, suggesting effective pollination within 300 m, but detected also pollination events over distances of up to 3 km. While these studies on pollination were carried out in isolated populations, where pollen can be transported easier with less barriers being present, a study by Heuertz et al. (2003) in a Romanian continuous ash woodland estimates pollination events within a distance of 14 m from female trees and pollen flow below 140 m distance. The low assignment rate for parent-pair analysis and moderate assignment rate for maternity analysis infer possible underestimation of seed and pollen dispersal within mixed continuous woodland and imply further analysis. However, it allows calculating ‘population differences’ in susceptibility between locally sired and incoming seedlings (with a good standardization of environmental effects). If technical errors and null alleles are a main reason for low assignment rates, then our conditions are conservative (in the sense that

more relaxed assignment conditions would lead to higher errors in parent-offspring assignments, so that correlations of disease tolerance would further be blurred).

7.2. *Variation in ash dieback susceptibility*

No significant relationship was found based on Spearman's rank correlation between damage intensity of parents and their offspring, whether for parent-offspring duos (offspring with one parent), nor for parent-offspring trios (offspring with both parents) (table 2).

Previous studies suggested genetically based variation as explanation for observed differences in susceptibility to dieback symptoms and assumed that these differences might be transferred across ash generations (Bakys et al. 2009, McKinney et al. 2011, Lobo et al. 2014). However, most of these studies were performed in clonal seed orchards or established progeny trials. Often the seedlings were raised in a nursery prior to planting (Kjaer et al. 2012) or the mother clones were grafted onto rootstocks (McKinney et al. 2011, Stener 2013). Therefore, progeny trials and seed orchards represent controlled environments and they often lack natural woodland conditions. In contrast, this study was conducted in natural ash stands. Lobo et al. (2014) estimated the heritability of damage intensity in two progeny trials in Denmark. Both trials included progenies from the same mother trees, but one trial was left unfenced and progenies were put under strong competition from vegetation and exposed to browsing animals, whereas the other was protected by fencing. Lower estimated heritability values of the unfenced (0.2) compared to the fenced site (0.42-0.53) indicated that expression of estimated heritability under heterogeneous natural conditions may be lower (Lobo et al. 2014). Johannser Kogel is also fenced, but the fence may be penetrable at some points, as boars were observed inside the fence during sampling. Pliura et al. (2014) tested clones from seven different sites from Lithuania exposed to different ash-dieback infection pressure. They calculated the genotype-environment interaction on phenotypic variation and found significant contributions of genetic variation in plasticity and reaction norms of clones across a range of infection

pressure environments (Pliura et al. 2014). This indicates the presence of variation of individual response to the disease across sites depending on site conditions (Pliura et al. 2011, 2014) or different levels of disease pressure. Results from previous studies, together with the current one suggest an overestimation of heritability in more controlled environments. Therefore, environmental conditions could be more relevant and need to be considered for inferences on susceptibility.

It seems from all cited studies that up to now, only a small fraction of ash individuals maintain potential resistance to the disease, as practically all trees are infected at some point. Kjaer et al. (2012) found high susceptibility and mortality among trees in two progeny trials in Denmark. They further estimated that only 1% of trees have the potential to pass lower susceptibility to their offspring. In three Lithuanian progeny trials almost 90% of the trees died during the observation period of 8 years (Pliura et al. 2011). In Austria, such high mortality rates have not yet been reported from observation sites (Heinze et al. submitted). In 50 ash dieback monitoring plots in Lower Austria, mean crown dieback intensity reached 18.1% in 2009 and 17.6% in 2010 (Kirisits and Freinschlag 2012). Three seed orchards in Austria showed moderate mean crown dieback intensity in 2011 (14.2%, 13.5% and 31%) and no clone was totally unaffected (Heinze et al. submitted). Crown and shoot dieback intensity was also relatively moderate in Siegenfeld and Johannser Kogel (66% and 72% of the individuals respectively had lower than 50% damage intensity). It is likely that the mature trees that were identified as parents did not express assessable resistance due to low infection pressure (especially for the higher crowns of thicker trees, supplementary figure 2), while infection pressure was higher for saplings close to the forest floor, where the presence of petioles and more humid conditions would favour high spore concentrations. This could be one reason for the low correlation found (although it may only impact the severity, but not so much the direction of susceptibility; healthier mature trees should then still produce healthier offspring, though the average damage class rating would be shifted between old trees and young saplings, which was the case in this study). Probably, the small sample size of parent-offspring trios ($N = 28$) and half-sib families (only nine mature trees produced more than two offspring) may have led to underestimation of present, but weak

correlations of damage intensity between parent and offspring for possible resistance.

Younger ash trees are assumed to be more affected by ash dieback, because disease is progressing more severely and rapidly than in older individuals (Heinze et al. submitted). Figure 4 indicates a possible relationship between diameter at breast height (DBH) and damage intensity, especially in Johannser Kogel, where older trees (higher DBH) tend to be less affected than younger ones (lower DBH).

Breeding values, which represent the average effect of the parent genotype, estimated from the performance of its offspring (McKinney et al. 2014), were calculated by Kjaer et al. (2012) in a Danish progeny trial. Only one of 101 tested mother trees had breeding values below 10% and was estimated to produce healthy offspring and four trees with breeding values below 20% were estimated to produce fairly healthy offspring. Another study carried out by McKinney et al. (2011) supported these findings, with only one of 39 tested clones exhibited breeding values below 10%. Distribution of breeding values for susceptibility in Kjaer et al. (2012) were normally distributed suggesting that possible resistance is based on expression of several genes rather than on one alone (McKinney et al. 2014).

Unfortunately, the genetic background of low susceptibility thus remains unclear and the robustness of the observed potential resistance in previous studies needs to be evaluated. It is uncertain whether resistance in single individuals could hinder massive decline of ash in Europe and if that resistance would sustain massive infection pressure at natural forest sites. However, by selection and breeding of non-susceptible trees throughout Europe might reduce the impact of ash dieback in the future.

8. Conclusion

In this study, no significant correlation could be found between ash dieback damage intensity of parent and offspring. I conclude that genetic resistance in parent trees is missing at both sites and that the weak statistical power of detecting

possible correlations is due to the low assignment rate of parent offspring trios or half-sib families. I further suggest that variation in susceptibility on a genetic basis might be more complex under natural heterogeneous conditions than in controlled environments. The robustness of the observed potential resistance in previous studies should therefore be critically evaluated. A scenario of large-scale decline of *Fraxinus excelsior* trees is becoming more and more likely. It will be important to identify and propagate healthy ash individuals throughout Europe, find out about the genetic mechanism of any resistance in more detail and implement a management program that accounts for these facets.

9. References

9.1. Articles

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9.2. Web Page

<https://www.wien.gv.at/umwelt/wald/erholung/lainzertiergarten/lebensraum/lage.html>, 05-20-2016

10. Figures and tables

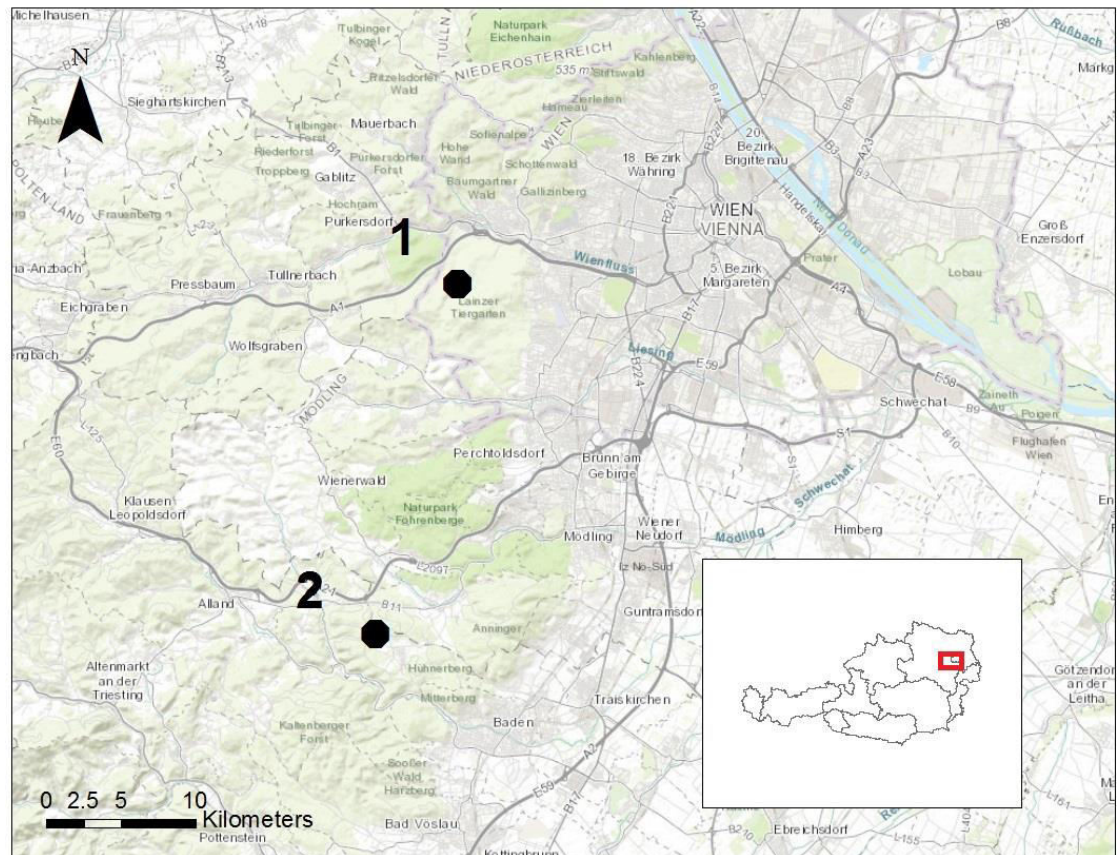


Figure 1: Location of the study sites in eastern Austria; 1: Johannser Kogel (Lainzer Tiergarten, southwest Vienna); 2: Siegenfeld (Heiligenkreuz, Wienerwald)

A



B

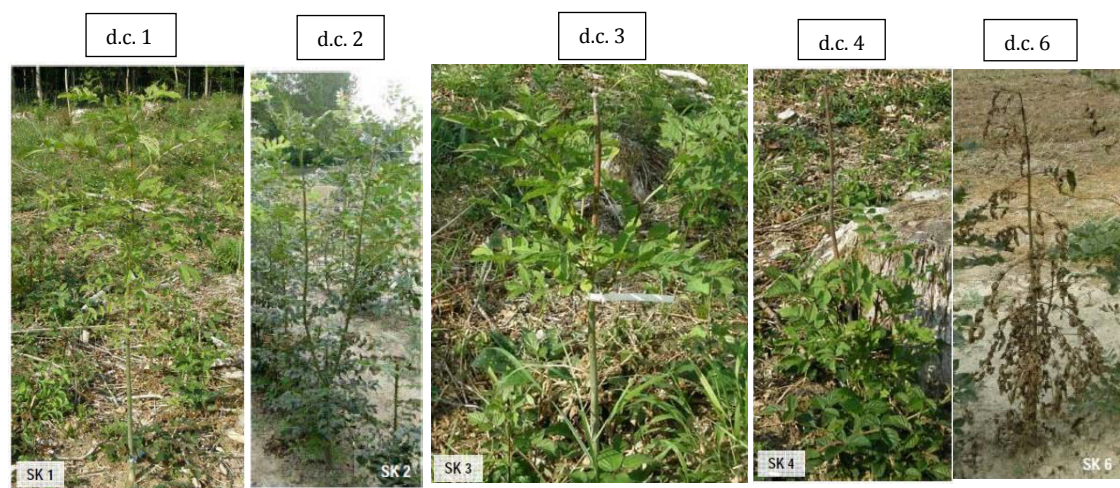


Figure 2: Illustration of the visually assessed damage classes (d.c.) (A) for mature trees, no tree was assessed for damage class 6 (photos from Siegenfeld, by Alexandra Wohlmuth); (B) for saplings (d.c. 5 is not shown) (Photos by T. Kirisits, IFFF-BOKU Wien)

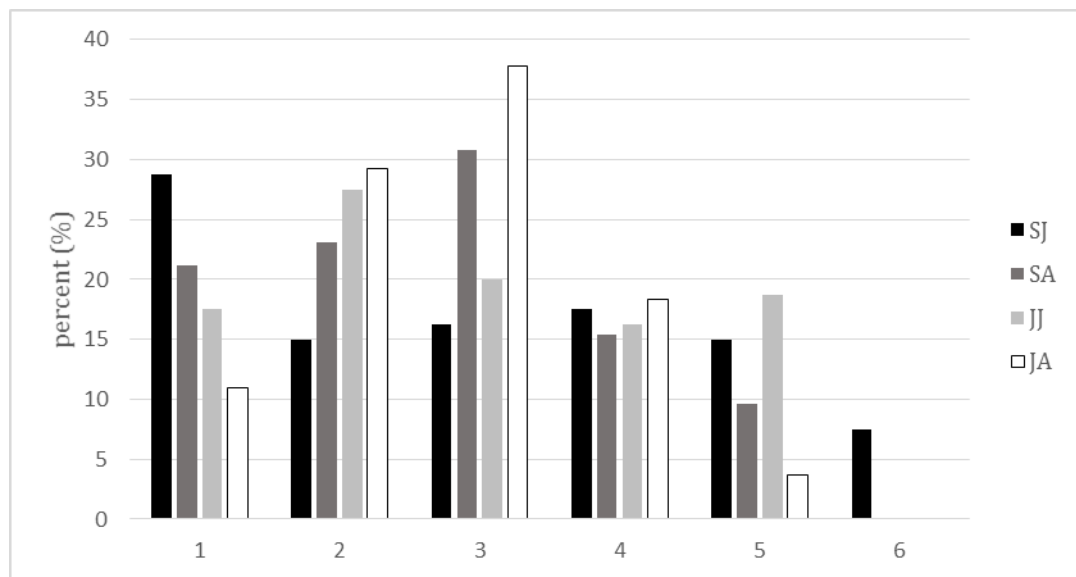


Figure 3. The percentage of ash individuals that belong to damage classes 1 – 6 shown separately for mature trees and saplings from each study site; SJ: saplings from Siegenfeld; SA: mature trees from Siegenfeld; JJ: saplings from Johannser Kogel; JA: mature trees from Johannser Kogel

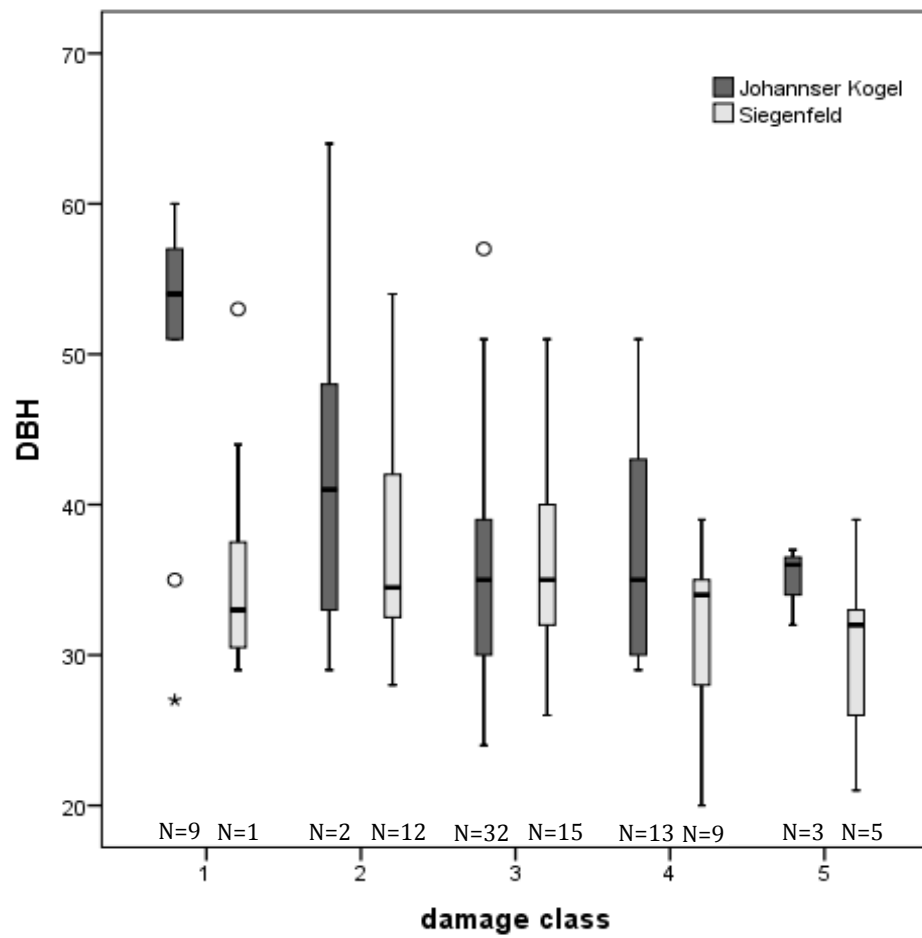


Figure 4. Boxplot of the assignment of mature ash trees of different size (measured by their diameter at breast height) to different damage classes at the study sites

Table 1: Information about the microsatellite loci used and the analyzed parameters. Legend: *number of alleles; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic information content; NE-1P: average non-exclusion for one candidate parent; NE-PP: average non-exclusion probability for one candidate parent; NE-PP: average non-exclusion probability for a candidate parent-pair; HW: Significance of deviation from Hardy-Weinberg equilibrium - NS = not significant, * = significant at 5% level, ** = significant at 1% level, *** = significant at 0.1% level; Null: Estimated null allele frequency*

Locus	Annealing temp. (C°)	analyzed parameters Johannser Kogel								analyzed parameters Siegenfeld							
		N	Ho	He	PIC	NE-1P	NE-PP	HW	Null	N	Ho	He	PIC	NE-1P	NE-PP	HW	Null
Femsatl-4	60	26	0.665	0.734	0.695	0.65	0.275	NS	0.052	21	0.734	0.808	0.787	0.53	0.162	NS	0.0437
Femsatl-10	50	41	0.745	0.895	0.884	0.347	0.067	*	0.0924	37	0.772	0.935	0.927	0.24	0.031	ND	0.0913
Femsatl-11	55	22	0.776	0.873	0.858	0.406	0.096	***	0.0589	19	0.789	0.883	0.869	0.384	0.085	NS	0.0561
Femsatl-12	55	17	0.376	0.737	0.701	0.646	0.268	***	0.3333	15	0.286	0.855	0.837	0.446	0.114	***	0.5006
Femsatl-16	62	7	0.547	0.526	0.454	0.858	0.588	NS	-0.0227	6	0.459	0.524	0.489	0.85	0.507	NS	0.0853
Femsatl-19	58	16	0.85	0.802	0.776	0.549	0.186	**	-0.0328	17	0.79	0.877	0.861	0.403	0.096	NS	0.0515
M230	57	37	0.844	0.891	0.881	0.349	0.066	*	0.0234	36	0.871	0.94	0.933	0.225	0.027	ND	0.0366
FR639485	55	9	0.638	0.583	0.524	0.816	0.496	NS	-0.051	8	0.677	0.654	0.601	0.757	0.405	NS	-0.0258
FR646655	60	8	0.696	0.695	0.647	0.719	0.36	NS	-0.0029	7	0.697	0.71	0.66	0.701	0.342	NS	0.008

Table 2. Spearman's rank-correlation analysis for offsprings for which both both parents were assigned (n = 26) (A), and for offspring for which one parent was assigned (n = 58) (B)

A

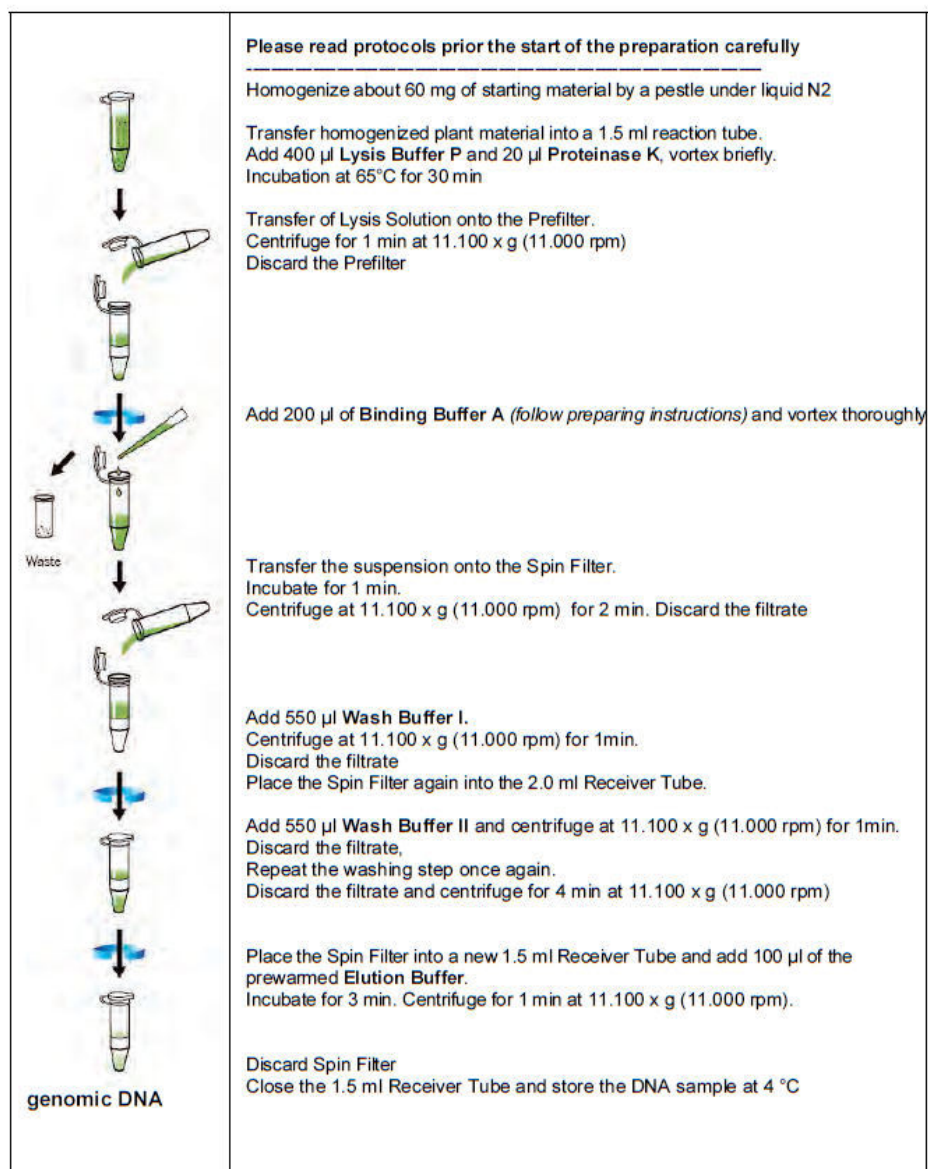
both parents assigned		mean damage parent-pair
Damage class offspring	Correlation Coefficient (r_s)	0.098
	P	0.318

B

one parent assigned		damage class one parent
Damage class offspring	Correlation Coefficient (r_s)	-0.082
	P	0.272

Appendix A:

Supplementary figure 1: DNA extraction scheme using Invisorb® Spin Plant Mini Kit.



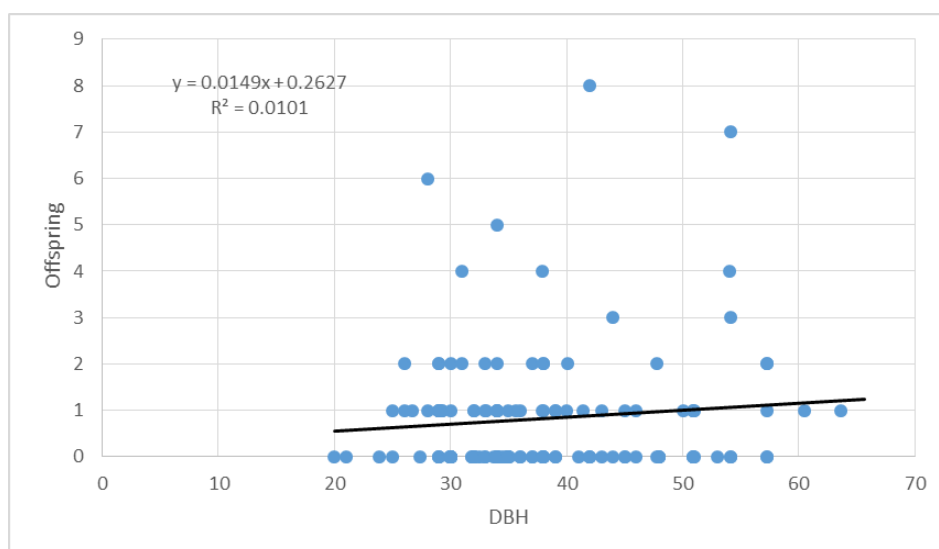
Appendix B:

Supplementary table 1: Number of offspring per mature ash tree separately shown for each damage class

offspring d.c. parent \ d.c.	1	2	3	4	5	6
1	1	6	4	3	2	0
2	10	8	4	5	7	0
3	7	7	5	11	9	0
4	3	9	0	1	4	0
5	1	1	0	1	1	0
6	0	0	0	0	0	0

Appendix C:

Supplementary figure 2: Diameter at breast height (DBH) of mature ash trees and the number of assigned offspring



Appendix D:

Supplementary table 2: Genotype data for each individual at each allele. Missing genotypes are encoded by 0.

Johannser Kogel

sapling	FEM 4a	FEM 4b	FEM 10a	FEM 10b	FEM 11a	FEM 11b	FEM 12a	FEM 12b	FEM 16a	FEM 16b	FEM 19a	FEM 19b	M230 a	M230 b	FR 63a	FR 63b	FR 64a	FR 64b
JJ1	116	118	184	256	175	185	186	186	194	194	141	148	207	233	300	306	180	186
JJ2	116	122	230	234	181	185	188	188	178	194	133	148	225	233	300	300	186	186
JJ3	112	122	164	302	185	185	188	208	178	180	138	155	241	255	304	304	180	180
JJ4	116	116	220	234	173	173	188	188	178	178	141	148	225	233	300	302	180	180
JJ5	116	142	186	186	185	193	188	188	178	194	141	141	233	253	304	314	180	186
JJ6	122	148	233	233	179	205	264	264	178	178	148	155	272	272	298	308	186	189
JJ7	118	152	186	186	189	220	200	214	178	194	130	141	209	223	300	306	189	189
JJ8	116	116	226	235	175	175	208	208	178	178	150	155	235	257	300	304	189	195
JJ9	118	126	186	220	175	195	186	202	178	178	150	155	233	233	300	308	186	189
JJ10	118	134	186	238	175	220	186	186	178	194	148	153	209	275	300	304	180	189
JJ11	118	118	186	334	175	175	0	0	194	194	141	148	209	233	306	306	183	189
JJ12	118	130	186	186	175	175	186	186	178	194	150	155	209	233	304	306	189	189
JJ13	116	118	186	189	207	213	190	190	178	194	141	141	209	275	296	300	189	189
JJ14	118	118	164	186	175	189	200	212	178	194	141	150	249	249	304	304	180	189
JJ15	116	116	186	252	185	220	186	186	178	194	155	158	207	223	304	304	189	189
JJ16	118	134	186	186	173	173	186	186	178	194	141	148	233	275	298	306	186	189
JJ17	118	120	183	186	173	173	186	186	180	180	141	148	223	243	304	304	189	195
JJ18	116	126	164	186	191	195	186	204	194	194	169	179	229	241	300	304	189	189
JJ19	118	136	234	234	173	181	188	188	176	178	150	153	223	275	300	304	180	189

JJ20	118	118	186	186	173	177	188	188	170	194	141	153	223	223	300	304	180	189
JJ21	148	154	254	370	179	181	188	204	178	178	141	153	209	243	304	304	189	195
JJ22	118	154	186	186	175	187	200	200	178	178	141	160	215	275	304	304	180	189
JJ23	118	142	164	224	173	193	186	186	178	178	141	148	217	263	304	304	180	189
JJ24	118	118	235	241	173	215	186	186	178	194	155	155	233	241	304	304	189	189
JJ25	116	116	234	330	173	195	188	188	178	178	130	141	221	233	304	304	180	189
JJ26	120	124	186	254	181	181	190	190	178	178	150	155	231	267	304	304	180	189
JJ27	116	116	186	243	173	195	196	196	178	178	141	155	257	275	304	304	180	189
JJ28	118	118	186	220	173	185	186	188	178	194	153	155	249	275	300	300	186	186
JJ29	116	118	234	235	175	197	188	188	178	194	146	155	267	275	304	304	186	189
JJ30	116	116	186	247	173	181	186	188	178	194	130	146	233	233	304	304	180	195
JJ31	116	116	235	260	175	195	188	188	178	178	141	148	209	261	300	304	189	192
JJ32	118	118	220	278	173	195	198	200	180	194	146	160	231	249	300	306	189	189
JJ33	116	116	185	247	173	195	186	208	178	178	143	148	217	217	300	300	189	189
JJ34	116	118	220	234	173	197	214	214	178	178	141	155	275	275	300	304	189	207
JJ35	118	118	164	328	175	191	214	214	178	194	141	146	237	249	300	306	189	189
JJ36	158	158	186	226	173	173	0	0	178	178	141	150	231	231	304	304	186	186
JJ37	116	148	230	230	173	181	186	186	178	180	0	0	251	275	300	304	186	189
JJ38	116	118	186	186	183	183	186	186	178	194	150	153	231	231	304	304	189	195
JJ39	118	118	185	370	183	209	200	200	180	180	148	155	225	225	298	300	180	189
JJ40	152	166	234	234	173	195	184	188	178	194	146	155	275	275	304	304	186	186
JJ41	116	116	220	234	173	181	188	190	178	194	141	141	251	275	300	300	189	189
JJ42	116	116	164	256	173	195	188	188	178	178	141	155	233	245	300	300	180	186
JJ43	118	120	184	241	175	175	188	188	178	194	130	141	233	233	300	304	189	189
JJ44	116	118	183	183	183	203	188	214	194	194	141	155	233	233	300	304	189	189
JJ45	118	122	234	234	175	175	186	186	178	194	141	146	209	233	300	304	180	189
JJ46	118	122	185	185	173	185	186	212	178	194	155	155	217	233	300	304	183	189

JJ47	116	118	186	238	179	185	186	186	178	194	141	155	217	267	300	304	180	189
JJ48	118	118	164	238	185	193	188	200	194	194	141	141	217	272	304	306	180	189
JJ49	116	118	164	247	183	183	208	212	178	180	141	155	215	223	298	304	195	195
JJ50	116	134	185	185	175	189	186	186	178	194	148	155	209	241	304	304	180	189
JJ51	116	116	183	234	185	211	188	188	178	178	141	150	233	257	298	300	186	189
JJ52	116	142	264	278	173	183	198	198	178	194	146	150	225	255	300	306	189	189
JJ53	116	124	164	235	175	175	198	198	178	178	146	146	233	255	308	316	189	195
JJ54	116	130	164	252	175	207	186	186	178	194	141	150	255	283	304	306	183	183
JJ55	116	122	250	256	181	185	186	198	178	194	141	155	207	275	304	306	186	186
JJ56	118	124	220	326	173	195	190	198	178	178	150	155	263	275	300	304	186	195
JJ57	122	146	250	338	175	175	188	202	178	194	133	155	253	275	304	304	189	189
JJ58	116	120	164	247	189	193	264	264	178	194	155	171	209	257	300	304	180	186
JJ59	116	136	164	254	175	175	198	198	178	194	146	146	219	249	306	306	186	189
JJ60	116	122	185	254	185	185	186	188	178	178	141	160	209	233	298	304	180	195
JJ61	136	152	185	185	173	197	186	202	178	194	141	155	233	233	300	304	189	195
JJ62	116	136	183	280	197	213	186	186	178	178	150	155	231	257	300	304	180	186
JJ63	116	116	234	234	185	185	186	188	178	178	138	155	233	247	304	304	180	195
JJ64	118	118	185	234	185	185	198	198	178	178	155	155	233	275	304	304	180	195
JJ65	118	118	235	235	185	185	188	188	178	178	148	155	219	233	304	306	189	189
JJ66	116	116	183	235	173	185	188	188	178	178	130	155	211	275	300	304	195	195
JJ67	118	144	186	235	175	185	200	200	178	178	155	155	275	275	300	304	186	195
JJ68	138	148	185	185	175	193	188	188	178	194	135	158	231	272	304	304	186	195
JJ69	116	118	186	186	185	195	186	204	178	178	141	155	213	275	300	300	186	186
JJ70	116	118	185	185	173	173	186	186	178	194	141	141	194	223	300	306	186	189
JJ71	116	118	186	186	191	220	186	190	178	180	130	155	233	255	304	304	189	189
JJ72	116	116	186	256	183	220	182	182	178	180	150	155	211	223	304	304	180	195
JJ73	116	116	185	185	173	181	186	186	178	194	141	148	223	253	304	306	180	189

JJ74	118	158	186	250	175	220	188	206	178	194	130	155	225	233	304	306	186	189
JJ75	116	142	185	218	173	220	186	200	178	180	141	155	233	233	304	306	189	189
JJ76	116	118	186	324	177	220	186	186	194	194	141	148	233	233	300	304	186	189
JJ77	116	118	164	186	175	220	186	186	180	180	141	141	223	267	300	304	189	195
JJ78	132	132	235	235	181	193	190	190	178	178	141	141	229	257	304	308	189	189
JJ79	116	118	185	258	173	181	0	0	178	180	153	155	233	253	298	304	189	195
JJ80	116	116	220	220	173	183	188	188	178	178	155	155	233	249	300	304	180	186
tree	FEM 4a	FEM 4b	FEM 10a	FEM 10b	FEM 11a	FEM 11b	FEM 12a	FEM 12b	FEM 16a	FEM 16b	FEM 19a	FEM 19b	M230 a	M230 b	FR 63a	FR 63b	FR 64a	FR 64b
JA1	116	118	185	233	185	193	186	188	180	194	146	155	233	275	300	304	189	189
JA2	116	116	185	220	185	215	186	212	178	192	153	155	257	275	304	304	180	189
JA3	116	118	189	234	177	185	188	202	178	194	143	155	233	261	300	304	180	189
JA4	116	118	185	233	185	193	186	186	178	178	138	141	247	275	304	304	183	189
JA5	116	160	185	234	175	183	186	186	178	194	141	146	231	275	304	304	186	189
JA6	116	146	234	334	173	193	186	186	178	194	141	150	219	275	300	304	186	189
JA7	116	118	234	326	175	185	188	204	194	194	141	141	233	233	298	304	189	189
JA8	118	122	234	250	173	195	186	196	178	178	148	155	233	251	300	304	180	186
JA9	116	118	185	234	173	189	182	188	178	178	141	150	247	275	300	304	180	189
JA10	116	116	183	234	175	175	188	188	178	194	155	158	233	245	304	304	189	195
JA11	118	140	185	245	181	185	186	186	178	194	141	153	223	233	300	304	189	189
JA12	116	116	164	234	185	195	186	202	178	194	141	141	249	275	300	304	180	189
JA13	116	118	185	234	173	181	186	186	178	194	141	155	223	275	300	304	189	189
JA14	118	158	234	338	173	191	186	214	178	178	155	158	227	275	300	304	180	189
JA15	116	136	226	234	185	195	186	186	178	178	141	155	253	275	300	304	186	189
JA16	116	122	164	185	185	191	188	208	178	178	146	155	243	275	304	304	189	189
JA17	118	122	164	234	173	181	188	188	178	178	141	155	235	235	300	304	180	189

JA18	118	124	185	185	175	183	186	188	178	194	141	141	192	233	304	306	186	189
JA19	114	116	164	185	185	189	186	188	178	178	148	155	251	275	300	304	180	195
JA20	116	148	185	324	175	200	188	188	178	194	135	141	275	275	300	304	180	195
JA21	116	118	189	234	181	185	186	186	178	194	155	155	229	233	300	304	180	189
JA22	116	118	185	230	181	185	182	186	178	194	141	141	257	275	300	304	189	189
JA23	116	116	234	256	181	185	188	264	178	194	141	146	229	233	300	304	189	189
JA24	116	140	183	234	185	191	186	186	178	194	138	155	194	233	300	304	180	189
JA25	116	126	164	234	173	177	186	212	178	178	141	150	235	275	304	304	180	201
JA27	116	164	183	185	175	175	186	186	178	194	138	155	231	275	300	304	186	189
JA28	116	116	185	235	177	177	186	186	178	178	153	155	253	275	300	304	186	189
JA29	118	122	186	189	175	179	188	188	178	194	141	148	275	294	298	304	189	207
JA30	116	166	185	250	181	185	186	186	178	194	141	150	233	275	300	304	180	186
JA31	116	122	185	185	173	181	188	200	178	194	155	160	263	305	304	304	180	195
JA32	118	118	185	324	175	175	188	200	178	178	146	155	275	275	300	304	189	189
JA33	116	116	185	247	173	179	188	202	178	178	141	155	231	275	300	304	189	189
JA34	118	138	234	234	181	185	188	188	178	178	153	155	251	275	300	304	180	189
JA35	116	118	234	256	175	183	186	216	176	194	138	155	215	233	304	304	180	189
JA36	118	150	185	250	175	191	188	190	178	194	141	148	215	275	300	304	180	189
JA37	116	122	185	247	185	193	186	204	176	194	148	155	275	287	300	304	189	189
JA38	118	118	234	256	173	193	186	192	178	178	133	141	237	237	304	306	180	186
JA39	118	118	234	250	185	185	186	198	178	194	141	155	219	275	300	304	186	189
JA40	118	118	185	185	173	200	186	186	178	178	141	155	209	209	304	304	189	207
JA41	118	134	185	234	185	193	186	186	176	194	141	155	253	275	300	304	180	189
JA42	116	116	234	234	173	179	186	186	178	178	155	155	217	275	302	304	180	207
JA43	118	126	185	241	175	185	188	188	178	194	141	150	233	275	304	304	189	189
JA44	116	116	234	234	175	175	186	186	178	178	141	158	233	243	300	304	180	207
JA45	116	116	234	234	175	185	188	188	178	178	141	148	223	275	304	306	186	189

JA46	116	118	234	252	185	197	188	188	178	194	155	155	209	275	304	304	189	189
JA47	118	132	185	185	173	185	188	188	178	178	141	155	209	233	304	306	180	189
JA48	114	116	185	330	177	185	188	206	178	178	141	141	209	233	300	304	186	189
JA49	116	116	234	234	185	215	186	200	178	194	141	146	225	233	304	304	180	186
JA50	116	116	185	260	185	195	188	188	178	178	141	155	261	275	300	304	189	189
JA51	118	118	164	185	175	175	186	186	178	194	141	141	215	233	304	304	180	189
JA52	116	122	234	234	175	179	186	186	178	178	141	148	225	275	304	304	180	186
JA53	116	152	234	266	175	185	186	186	178	194	155	179	225	233	304	304	180	180
JA54	116	154	185	254	181	185	188	200	178	194	141	160	209	275	304	304	180	195
JA55	114	116	234	274	173	185	186	186	178	194	141	148	233	249	304	304	180	183
JA56	118	122	234	247	175	175	186	186	178	194	148	155	225	275	304	304	180	186
JA57	118	118	183	234	173	193	188	188	178	194	130	141	209	233	304	304	180	189
JA58	116	118	234	271	181	185	188	188	178	194	141	143	233	233	300	304	180	180
JA59	116	148	278	278	195	213	186	186	178	178	148	155	231	255	300	300	186	186
JA60	118	136	185	185	185	197	186	186	178	194	141	150	233	257	304	304	180	195
JA61	118	128	234	234	185	185	188	188	178	178	150	155	217	275	304	304	189	189
JA62	118	118	183	185	185	203	188	188	194	194	141	158	235	275	300	304	189	189
JA63	116	116	164	185	173	173	186	212	178	194	141	148	245	275	300	304	180	186
JA64	118	152	220	234	185	195	186	188	178	194	146	155	245	275	300	304	186	189
JA65	118	124	185	247	173	200	186	186	178	188	141	148	225	233	300	304	180	189
JA66	118	118	185	247	175	175	182	188	178	178	141	150	217	233	304	304	180	189
JA67	116	116	234	250	185	185	188	188	178	178	141	148	233	280	296	304	189	195
JA68	116	158	185	238	175	177	188	188	178	194	148	155	233	275	304	304	180	186
JA69	116	116	185	316	175	185	188	188	194	194	135	141	261	275	298	304	180	189
JA70	116	116	183	235	173	183	200	200	178	178	141	141	0	0	296	302	180	189
JA71	116	124	234	254	173	181	186	190	178	194	141	155	231	275	300	304	186	189
JA72	116	118	234	328	173	185	188	196	178	178	146	155	215	233	304	304	180	195

JA73	118	158	164	234	173	173	0	0	178	178	143	155	221	231	0	180	189
JA74	116	118	164	234	173	195	186	186	178	178	150	155	275	275	300	304	189
JA75	116	118	220	234	175	175	186	186	178	178	141	158	231	275	300	304	189
JA76	118	122	234	278	187	187	186	186	178	178	155	158	233	253	300	304	186
JA77	116	118	164	185	185	215	186	214	170	194	141	153	217	233	304	304	186
JA78	116	116	164	185	181	185	186	216	180	194	141	153	233	253	300	304	189
JA79	116	118	185	214	173	185	186	202	178	178	148	155	257	275	304	304	189
JA80	116	118	185	185	173	220	186	186	180	194	141	155	223	233	304	306	189
JA81	136	136	164	164	173	177	186	186	170	176	148	153	223	229	300	304	189
JA82	118	126	186	186	173	195	186	186	178	194	155	179	229	233	300	304	189

Siegenfeld

sapling	FEM 4a	FEM 4b	FEM 10a	FEM 10b	FEM 11a	FEM 11b	FEM 12a	FEM 12b	FEM 16a	FEM 16b	FEM 19a	FEM 19b	M230 a	M230 b	FR 63a	FR 63b	FR 64a	FR 64b
SJ1	114	114	164	330	173	195	188	188	178	178	148	153	231	247	304	304	186	195
SJ2	118	118	185	266	177	193	200	200	178	178	150	150	219	265	300	300	189	192
SJ3	116	118	183	186	183	193	184	200	178	178	150	158	235	235	300	304	189	189
SJ4	116	122	220	234	173	173	186	186	180	194	150	158	213	249	304	304	186	189
SJ6	116	126	220	233	181	195	200	202	178	194	146	153	233	237	304	306	180	186
SJ7	116	124	233	245	179	195	196	196	178	178	130	153	237	251	300	304	186	189
SJ8	122	144	226	238	173	173	214	214	178	178	148	153	233	233	300	306	186	195
SJ9	116	116	164	164	173	183	188	212	178	178	150	150	209	235	304	310	186	189
SJ10	116	116	164	183	175	185	200	200	178	178	141	153	247	253	300	304	189	189
SJ11	126	148	183	342	173	173	186	186	178	188	143	153	225	233	300	304	189	189
SJ12	116	122	235	247	183	189	188	188	188	194	146	153	229	247	300	300	186	189
SJ13	118	118	194	230	173	185	204	204	178	180	148	148	213	272	306	306	180	189

SJ14	116	116	116	164	332	175	189	186	186	188	194	141	153	233	247	304	308	189	189
SJ16	128	128	186	186	266	209	215	202	202	178	178	130	179	237	251	300	304	186	189
SJ17	116	116	164	233	185	195	195	200	200	188	194	148	153	225	245	300	304	186	186
SJ18	116	118	233	247	181	193	193	190	202	194	194	141	148	215	275	304	306	186	195
SJ19	116	118	186	235	181	226	186	186	186	178	180	148	148	233	272	300	304	189	192
SJ21	116	120	164	260	173	183	183	200	202	178	178	130	130	207	280	300	306	186	186
SJ22	116	132	186	247	173	195	195	200	200	178	194	150	155	237	283	300	304	186	189
SJ23	116	122	260	310	173	195	195	186	186	178	188	150	158	227	251	300	300	189	189
SJ24	142	142	186	234	173	173	173	204	212	180	180	150	150	255	272	300	306	186	189
SJ25	116	116	183	258	195	195	195	198	198	178	194	148	155	227	280	300	310	186	192
SJ26	116	122	258	258	183	183	183	188	188	178	178	148	150	207	213	300	304	186	186
SJ27	118	126	184	220	175	215	215	198	198	178	178	148	150	209	219	300	300	186	189
SJ28	118	122	0	0	195	195	195	0	0	178	178	146	148	194	194	304	306	186	195
SJ29	116	116	226	235	175	175	175	202	202	194	194	148	148	209	223	304	304	189	195
SJ30	116	152	184	184	183	183	183	200	200	178	178	148	150	272	283	298	310	186	189
SJ31	116	148	185	185	185	195	195	0	0	178	188	130	150	233	283	300	310	186	186
SJ34	122	144	164	183	183	173	195	184	184	178	178	153	158	209	263	300	306	189	189
SJ35	118	122	184	184	179	226	186	186	186	178	180	148	148	280	289	300	300	186	189
SJ36	116	116	183	332	175	195	195	200	200	178	188	146	158	225	225	300	300	186	189
SJ37	134	148	183	330	175	193	193	182	200	178	188	138	150	231	247	300	304	180	207
SJ38	120	128	180	185	175	195	195	186	186	178	178	141	150	245	251	300	304	180	189
SJ39	142	152	180	180	0	0	0	0	0	178	180	150	155	229	233	304	310	186	186
SJ40	118	138	164	185	181	209	209	198	198	178	178	130	155	209	229	300	304	189	189
SJ41	118	124	164	185	173	191	191	204	204	178	178	135	138	207	207	300	308	183	186
SJ42	116	118	212	235	187	226	226	186	186	178	180	143	148	209	272	300	304	186	192
SJ43	116	128	183	214	173	173	173	200	200	178	180	141	185	245	249	304	304	189	195
SJ44	116	124	164	183	183	187	187	200	204	178	180	153	179	227	247	300	300	186	195

SJ45	116	142	185	330	175	175	190	190	178	180	158	158	213	225	300	306	186	189
SJ46	114	116	164	234	181	181	202	202	180	194	148	153	237	245	300	306	180	186
SJ47	118	142	186	235	183	226	190	190	180	180	158	158	272	280	300	306	180	192
SJ48	118	122	183	185	181	226	186	186	178	178	141	148	251	280	300	300	189	192
SJ49	116	148	330	330	175	195	200	200	178	178	130	150	225	247	300	304	189	195
SJ50	116	148	310	310	195	195	186	186	178	178	130	141	225	233	300	308	189	195
SJ51	128	148	230	330	173	189	186	214	178	178	130	153	245	255	300	308	189	189
SJ53	134	148	271	271	195	195	200	200	178	188	146	153	225	243	300	304	186	186
SJ54	116	122	238	238	183	209	188	204	178	178	146	150	229	233	304	306	186	189
SJ56	118	134	185	230	173	193	182	190	178	178	148	153	213	225	304	306	189	207
SJ57	114	122	186	238	173	209	186	202	178	178	148	148	237	251	300	304	189	195
SJ59	116	116	185	243	179	183	204	204	178	178	148	158	225	283	304	304	189	189
SJ60	144	144	164	220	195	195	0	0	178	180	150	158	233	263	300	300	189	195
SJ61	114	116	238	264	175	175	186	204	178	178	138	150	233	233	304	308	186	186
SJ62	116	122	233	238	173	183	202	202	178	178	130	158	241	255	304	304	180	189
SJ63	114	116	180	183	175	183	212	214	178	178	138	141	217	243	300	306	186	186
SJ64	116	116	164	238	181	195	186	186	178	180	141	148	209	247	300	304	189	195
SJ65	126	148	180	185	181	193	200	200	178	178	150	185	233	265	300	304	180	192
SJ66	118	118	186	220	181	195	188	188	178	180	148	148	209	265	300	304	189	189
SJ67	116	160	235	247	185	197	182	182	178	178	148	171	233	233	296	300	192	195
SJ68	114	118	186	254	181	207	200	200	178	178	141	148	209	233	300	300	186	192
SJ69	118	122	183	186	183	193	200	204	178	194	148	148	233	287	300	304	186	189
SJ70	122	148	278	285	177	183	186	186	178	178	153	153	233	255	300	306	180	186
SJ71	116	122	235	235	175	185	182	196	178	178	146	150	231	267	296	304	186	192
SJ72	116	118	186	220	193	230	204	204	178	178	148	158	251	265	300	300	189	189
SJ73	114	134	183	186	173	195	200	200	178	188	135	153	217	225	300	300	186	195
SJ74	118	118	226	226	183	197	204	204	178	178	150	158	233	233	300	304	195	195

SJ75	134	142	164	180	177	177	186	186	178	178	130	148	217	229	300	308	186	189
SJ76	148	170	226	226	173	195	200	200	178	188	150	153	225	233	300	304	195	195
SJ77	148	148	212	212	195	195	182	182	178	178	130	141	233	237	300	300	180	195
SJ78	116	128	184	186	195	209	200	200	178	178	148	150	209	255	300	300	180	189
SJ79	116	148	164	164	183	195	184	200	188	192	153	153	225	243	300	300	186	189
SJ80	118	118	184	184	179	195	200	200	180	188	141	158	225	247	298	300	189	189
tree	FEM 4a	FEM 4b	FEM 10a	FEM 10b	FEM 11a	FEM 11b	FEM 12a	FEM 12b	FEM 16a	FEM 16b	FEM 19a	FEM 19b	M230 a	M230 b	FR 63a	FR 63b	FR 64a	FR 64b
	122	148	184	186	175	195	200	202	178	178	141	169	225	257	300	300	189	189
SA1	116	116	183	233	175	183	202	202	178	188	141	155	225	249	300	304	180	189
SA2	122	148	186	330	175	181	200	214	0	0	143	160	231	247	300	304	0	0
SA3	118	122	226	235	175	175	0	0	0	0	150	155	233	251	300	300	0	0
SA4	116	116	330	330	181	195	186	186	188	192	148	153	225	231	300	304	186	189
SA5	116	148	334	334	185	195	190	190	178	188	141	153	247	267	300	306	189	189
SA6	124	148	183	183	175	207	186	186	188	194	141	141	247	267	300	304	186	207
SA7	116	148	233	330	181	191	200	200	178	188	141	153	225	237	300	306	180	189
SA8	116	148	164	183	175	195	200	200	188	188	153	179	209	247	300	306	186	186
SA9	116	116	164	183	173	193	200	200	178	188	141	163	243	247	300	304	186	186
SA10	116	118	164	224	181	189	214	214	178	178	148	148	217	223	300	306	180	186
SA11	118	148	183	280	191	195	200	206	178	188	141	141	219	247	298	300	186	207
SA12	116	118	164	185	183	195	212	212	178	178	150	155	209	257	300	310	186	189
SA13	148	148	164	183	175	195	212	212	178	188	153	169	225	225	300	300	189	189
SA14	116	118	183	183	177	177	186	200	180	188	153	160	215	247	300	304	186	186
SA15	116	118	183	234	177	177	202	212	178	178	148	148	209	233	300	306	186	189
SA16	116	116	183	185	181	195	200	200	178	194	148	150	283	283	304	310	186	189
SA17	116	116	183	226	175	195	204	212	178	178	150	160	209	219	300	300	186	189
SA18	116	116	183	226	175	195	204	212	178	178	150	160	209	219	300	300	186	189

SA19	116	126	185	185	175	175	198	212	178	178	148	150	209	243	300	300	186	189
SA21	116	154	185	238	173	195	200	212	178	178	148	158	237	280	308	310	186	195
SA22	116	124	183	214	179	195	200	202	178	178	153	185	225	283	300	304	180	189
SA23	116	132	183	330	175	179	200	200	178	178	141	160	211	247	300	304	189	195
SA24	130	140	164	189	181	183	182	188	178	178	158	158	194	227	304	304	186	189
SA25	116	122	238	334	175	195	214	214	180	188	153	153	225	225	300	306	189	189
SA26	128	128	185	238	175	209	186	186	178	178	130	148	251	255	300	300	180	189
SA27	116	118	185	185	173	195	200	200	178	178	148	148	209	213	300	304	186	189
SA28	116	116	183	226	175	195	200	200	178	178	130	150	209	237	300	310	186	189
SA29	116	122	238	334	175	195	214	214	180	188	153	153	225	225	300	306	189	189
SA30	116	116	310	330	175	195	186	186	178	178	141	158	225	251	300	308	186	189
SA31	116	148	330	338	195	195	186	264	188	188	141	153	225	259	300	304	186	189
SA32	116	148	247	330	175	189	214	214	178	188	150	153	247	247	300	308	186	189
SA33	116	118	164	233	181	185	202	202	178	194	148	158	245	272	304	306	180	186
SA34	114	148	330	330	195	195	200	200	178	188	130	153	225	233	300	300	186	195
SA35	116	116	164	338	183	205	184	184	178	192	148	153	225	243	300	306	189	189
SA36	114	134	183	185	175	193	182	188	178	178	138	153	209	225	304	304	186	207
SA37	114	148	220	328	175	181	200	200	176	188	141	150	237	247	300	300	180	189
SA38	116	148	183	183	175	179	182	182	178	178	150	160	247	255	300	304	186	189
SA39	116	128	230	230	173	187	186	186	178	178	130	148	255	267	300	304	189	189
SA40	116	122	235	278	183	209	188	188	178	194	146	158	229	237	300	300	189	195
SA41	116	116	164	185	183	191	202	202	178	194	141	153	209	249	300	304	186	186
SA42	122	148	183	245	183	195	200	200	178	188	143	143	225	233	300	300	180	189
SA43	116	116	226	238	173	183	204	204	178	178	150	150	233	233	306	306	186	195
SA44	116	118	183	234	179	195	190	200	180	188	141	158	225	233	298	300	186	189
SA45	118	118	186	186	181	193	200	200	178	178	148	150	233	265	300	300	189	192
SA46	116	148	185	234	183	187	182	182	178	178	148	150	231	231	296	304	189	192

SA47	126	128	183	183	179	193	190	202	178	178	143	143	209	227	304	304	189	189
SA48	116	118	183	264	175	195	186	186	178	188	135	141	251	251	300	300	189	207
SA49	116	118	183	235	175	226	200	200	178	180	148	158	272	280	300	300	186	192
SA50	122	122	238	238	173	209	188	188	178	188	130	158	237	255	300	304	180	180
SA51	112	116	183	266	175	175	206	258	178	178	141	150	237	280	300	316	186	186
SA52	116	122	183	185	183	195	200	202	178	188	141	146	225	261	300	300	189	189
SA53	118	142	185	226	173	197	188	214	178	194	153	185	217	225	304	306	186	189

Appendix E:

Supplementary table 3: Results of maternity analysis for Johannser Kogel (A), Siegenfeld (B) and parent-pair analysis for Johannser Kogel (C), Siegenfeld (D)

A							B						
sapling	NE-P1	p1	loci-mm	Pair LOD	Pair Delta	Pair conf.	sapling	NE-P1	p1	loci-mm	Pair LOD	Pair Delta	Pair conf.
JJ1	1.08E-03	JA18	1	-1.02	0.00		SJ1	7.67E-05	SA34	3	-5.17	0.00	
JJ2	8.91E-04	JA45	2	-3.56	0.00		SJ2	4.67E-05	SA45	1	7.10	7.10	*
JJ3	3.29E-05	JA16	2	-1.45	0.00		SJ3	2.95E-04	SA45	1	2.37	2.37	*
JJ4	2.49E-03	JA58	1	-0.62	0.00		SJ4	2.85E-05	SA41	4	-10.50	0.00	
JJ5	4.18E-03	JA58	1	-1.61	0.00		SJ6	1.07E-03	SA8	0	6.09	5.89	*
JJ6	5.05E-09	JA29	3	-5.63	0.00		SJ7	3.58E-05	SA28	2	-4.66	0.00	
JJ7	9.03E-05	JA80	2	-3.54	0.00		SJ8	8.13E-05	SA53	2	-1.95	0.00	
JJ8	2.74E-05	JA16	3	-7.97	0.00		SJ9	2.59E-04	SA13	0	9.83	9.83	*
JJ9	3.98E-03	JA82	0	5.61	5.61	*	SJ10	2.13E-03	SA23	0	5.10	5.10	*
JJ10	3.92E-03	JA56	1	-1.13	0.00		SJ11	5.54E-04	SA42	2	-1.55	0.00	
JJ11	7.31E-04	JA18	1	-0.12	0.00		SJ12	6.38E-05	SA40	0	11.81	8.11	*
JJ12	4.69E-03	JA82	1	-1.70	0.00		SJ13	3.49E-06	SA33	2	-0.05	0.00	
JJ13	4.34E-05	JA36	2	-4.65	0.00		SJ14	3.49E-04	SA32	2	-0.09	0.00	
JJ14	2.63E-04	JA51	2	-2.95	0.00		SJ16	4.36E-06	SA26	2	0.20	0.20	*

JJ15	1.47E-03	JA80	1	0.53	0.53	+	SJ17	1.26E-03	SA33	1	4.80	4.17	*
JJ16	2.29E-03	JA80	1	-2.02	0.00		SJ18	4.16E-06	SA33	1	4.54	4.54	*
JJ17	2.01E-04	JA80	1	0.51	0.51	+	SJ19	7.49E-04	SA49	1	6.42	3.20	*
JJ18	1.35E-05	JA82	0	12.48	12.48	*	SJ21	1.82E-04	SA41	2	-4.16	0.00	
JJ19	3.75E-03	JA34	0	6.11	2.77	*	SJ22	1.39E-03	SA17	1	2.47	2.47	*
JJ20	6.61E-04	JA81	3	-1.43	0.00		SJ23	1.87E-04	SA30	0	7.28	7.28	*
JJ21	1.19E-05	JA54	0	9.45	9.45	*	SJ24	3.01E-06	SA43	4	-9.42	0.00	
JJ22	6.95E-04	JA54	2	0.95	0.95	+	SJ25	8.43E-05	SA17	2	-2.52	0.00	
JJ23	8.09E-04	JA51	2	-4.18	0.00		SJ26	1.14E-05	SA27	3	-7.80	0.00	
JJ24	1.42E-03	JA82	1	-1.48	0.00		SJ27	5.18E-05	SA19	1	5.83	5.83	*
JJ25	7.72E-03	JA57	1	1.02	0.23	+	SJ28	6.78E-04	SA27	1	-1.74	0.00	
JJ26	7.06E-06	JA71	0	10.26	10.26	*	SJ29	1.91E-05	SA41	4	-8.83	0.00	
JJ27	3.94E-04	JA72	2	-2.76	0.00		SJ30	2.80E-05	SA17	2	-0.52	0.00	
JJ28	3.17E-03	JA64	0	4.80	4.80	*	SJ31	2.64E-03	SA17	0	7.36	5.97	*
JJ29	1.13E-02	JA46	0	5.92	5.71	*	SJ34	1.07E-04	SA35	3	-5.19	0.00	
JJ30	2.20E-03	JA72	1	0.29	0.29	+	SJ35	2.19E-05	SA49	2	-0.66	0.00	
JJ31	6.57E-04	JA50	0	10.78	10.78	*	SJ36	2.48E-03	SA52	0	6.16	1.75	*
JJ32	2.11E-05	JA64	2	-2.84	0.00		SJ37	3.03E-04	SA36	1	7.35	5.20	*
JJ33	2.87E-04	JA65	2	-3.73	0.00		SJ38	1.88E-04	SA26	1	4.89	4.89	*
JJ34	1.42E-03	JA14	0	4.09	2.79	*	SJ39	8.81E-05	SA13	3	-7.38	0.00	
JJ35	3.05E-05	JA14	3	-6.16	0.00		SJ40	2.70E-05	SA13	2	-2.60	0.00	

JJ36	4.05E-05	JA73	3	-4.03	0.00		SJ41	3.59E-06	SA27	3	-9.55	0.00	
JJ37	1.54E-03	JA22	0	4.19	4.19	*	SJ42	1.10E-04	SA49	1	5.88	5.88	*
JJ38	1.44E-04	JA5	2	-2.40	0.00		SJ43	1.60E-04	SA22	2	-1.67	0.00	
JJ39	3.53E-06	JA65	3	-7.16	0.00		SJ44	1.25E-03	SA15	1	-0.43	0.00	
JJ40	1.88E-04	JA64	0	8.52	8.52	*	SJ45	1.93E-04	SA30	1	0.37	0.37	*
JJ41	5.09E-03	JA71	0	5.35	3.78	*	SJ46	7.53E-05	SA33	0	10.30	10.30	*
JJ42	2.57E-03	JA63	1	3.05	3.05	*	SJ47	1.40E-06	SA49	1	8.07	8.07	*
JJ43	4.44E-04	JA43	0	6.63	6.63	*	SJ48	5.32E-04	SA49	1	3.44	1.81	*
JJ44	3.81E-04	JA62	1	5.31	4.67	*	SJ49	2.95E-03	SA34	0	7.98	5.68	*
JJ45	1.23E-02	JA52	1	0.53	0.31	+	SJ50	2.59E-04	SA30	0	9.40	9.40	*
JJ46	1.37E-02	JA1	0	1.30	0.34	+	SJ51	1.16E-04	SA39	0	10.47	5.32	*
JJ47	3.90E-03	JA42	1	1.26	1.26	+	SJ53	4.37E-05	SA34	1	1.70	1.70	*
JJ48	4.39E-04	JA77	1	0.75	0.75	+	SJ54	3.00E-04	SA43	0	9.19	8.20	*
JJ49	1.57E-05	JA72	3	-7.56	0.00		SJ56	2.83E-04	SA36	0	10.71	8.85	*
JJ50	3.30E-03	JA19	1	0.21	0.21	+	SJ57	4.14E-04	SA26	1	2.99	2.99	*
JJ51	5.40E-03	JA23	0	1.48	0.86	+	SJ59	2.01E-04	SA17	2	-2.37	0.00	
JJ52	7.75E-06	JA59	4	-9.74	0.00		SJ60	1.91E-04	SA44	2	-4.95	0.00	
JJ53	3.87E-06	JA16	4	-12.35	0.00		SJ61	2.20E-04	SA43	2	-0.22	0.00	
JJ54	2.19E-05	JA55	3	-8.22	0.00		SJ62	7.18E-05	SA50	1	6.28	6.28	*
JJ55	1.96E-03	JA30	0	3.87	1.54	+	SJ63	1.98E-04	SA19	2	-2.33	0.00	
JJ56	2.56E-04	JA71	1	1.36	1.36	+	SJ64	3.47E-03	SA30	2	-5.78	0.00	

JJ57	1.51E-04	JA36	2	-4.41	0.00		SJ65	1.38E-04	SA45	2	0.47	0.47	*
JJ58	2.49E-05	JA19	2	-4.59	0.00		SJ66	1.57E-04	SA45	1	4.28	4.28	*
JJ59	3.59E-06	JA45	4	-12.10	0.00		SJ67	1.10E-05	SA46	3	-4.12	0.00	
JJ60	9.61E-03	JA54	0	8.19	8.19	*	SJ68	3.62E-04	SA45	0	8.91	8.91	*
JJ61	1.82E-03	JA60	0	8.36	8.36	*	SJ69	2.38E-03	SA45	0	6.05	6.05	*
JJ62	9.03E-05	JA60	1	4.80	1.36	+	SJ70	3.60E-05	SA42	3	-8.02	0.00	
JJ63	6.12E-03	JA24	0	4.63	0.41	+	SJ71	2.36E-05	SA46	2	2.24	2.24	*
JJ64	1.18E-03	JA39	1	1.94	1.25	+	SJ72	6.42E-05	SA45	1	4.77	4.77	*
JJ65	1.06E-03	JA47	1	0.49	0.49	+	SJ73	6.73E-04	SA34	1	3.43	3.43	*
JJ66	8.63E-04	JA19	1	-0.45	0.00		SJ74	1.45E-05	SA43	2	3.09	1.68	*
JJ67	7.42E-04	JA32	2	-1.34	0.00		SJ75	1.40E-05	SA11	3	-8.08	0.00	
JJ68	9.91E-06	JA20	1	5.48	5.48	*	SJ76	2.38E-04	SA34	1	2.94	2.94	*
JJ69	2.76E-03	JA15	1	0.27	0.27	+	SJ77	5.74E-06	SA34	2	-1.64	0.00	
JJ70	2.16E-03	JA80	0	6.24	0.93	+	SJ78	1.92E-03	SA26	2	1.11	1.11	*
JJ71	1.72E-03	JA80	1	0.18	0.18	+	SJ79	7.87E-04	SA35	0	12.37	9.39	*
JJ72	1.91E-05	JA80	2	-2.62	0.00		SJ80	1.52E-04	SA44	1	6.34	6.34	*
JJ73	5.46E-03	JA80	0	5.92	1.56	+							
JJ74	5.24E-03	JA68	1	-0.04	0.00								
JJ75	1.22E-02	JA80	0	7.13	7.13	*							
JJ76	1.91E-03	JA80	1	-0.96	0.00								
JJ77	4.40E-04	JA80	1	2.23	2.23	+							

JJ78	5.92E-07	JA22	3	-7.66	0.00	
JJ79	2.18E-02	JA78	0	5.76	4.09	*
JJ80	1.11E-03	JA72	1	-1.86	0.00	

C								D							
sapling	NE-PP	p1	p2	trio LOD	trio Delta	trio conf.		sapling	NE-PP	p1	p2	trio LOD	trio Delta	trio conf.	
JJ1	1.78E-09	JA30	JA56	-4.65	0.00			SJ1	1.77E-10	SA3	SA10	-9.18	0.00		
JJ2	3.89E-10	JA17	JA22	-7.77	0.00			SJ2	3.04E-11	SA4	SA45	0.41	0.41	+	
JJ3	1.72E-14	JA1	JA16	-11.61	0.00			SJ3	6.94E-10	SA2	SA45	-2.47	0.00		
JJ4	1.66E-08	JA65	JA70	-0.99	0.00			SJ4	1.88E-11	SA4	SA44	-15.39	0.00		
JJ5	6.11E-10	JA15	JA57	-7.70	0.00			SJ6	1.72E-10	SA17	SA33	-5.99	0.00		
JJ6	2.52E-19	JA29	JA59	-10.59	0.00			SJ7	1.81E-12	SA22	SA28	-2.88	0.00		
JJ7	4.78E-12	JA9	JA80	-11.78	0.00			SJ8	9.70E-11	SA4	SA43	-3.86	0.00		
JJ8	1.09E-11	JA17	JA60	-8.54	0.00			SJ9	8.20E-10	SA10	SA13	1.16	1.16	+	
JJ9	7.17E-10	JA8	JA43	-2.24	0.00			SJ10	2.53E-08	SA6	SA23	-2.67	0.00		
JJ10	3.93E-09	JA40	JA41	-4.06	0.00			SJ11	2.73E-10	SA14	SA47	-2.73	0.00		
JJ11	8.65E-10	JA18	JA29	-7.88	0.00			SJ12	9.97E-12	SA32	SA40	18.91	14.37	*	
JJ12	3.18E-08	JA18	JA40	-9.02	0.00			SJ13	7.27E-14	SA27	SA33	-1.41	0.00		
JJ13	2.43E-12	JA36	JA70	-4.40	0.00			SJ14	1.40E-10	SA2	SA32	-5.38	0.00		
JJ14	4.97E-10	JA9	JA51	-2.70	0.00			SJ16	1.20E-13	SA26	SA47	-11.54	0.00		
JJ15	2.83E-09	JA75	JA80	-4.59	0.00			SJ17	8.28E-09	SA9	SA33	7.56	1.45	+	
JJ16	2.35E-08	JA8	JA41	-4.99	0.00			SJ18	5.95E-14	SA33	SA41	-7.80	0.00		
JJ17	7.35E-11	JA80	JA81	-12.29	0.00			SJ19	1.33E-09	SA45	SA49	10.06	9.56	*	
JJ18	4.26E-14	JA16	JA82	1.30	1.30	-		SJ21	1.19E-11	SA21	SA41	-12.22	0.00		
JJ19	6.18E-09	JA34	JA81	4.35	0.13	-		SJ22	3.98E-10	SA17	SA28	-5.02	0.00		
JJ20	9.31E-10	JA13	JA81	2.03	0.68	-		SJ23	4.15E-10	SA4	SA30	-1.69	0.00		
JJ21	2.86E-14	JA16	JA54	-5.79	0.00			SJ24	2.96E-13	SA13	SA43	-12.14	0.00		

JJ22	3.83E-11	JA51	JA54	2.28	2.28	+	SJ25	1.76E-12	SA13	SA17	-7.19	0.00
JJ23	1.49E-10	JA4	JA63	-5.21	0.00		SJ26	1.16E-11	SA4	SA27	-10.74	0.00
JJ24	6.73E-09	JA2	JA82	-5.75	0.00		SJ27	4.43E-12	SA4	SA19	-0.81	0.00
JJ25	1.04E-08	JA48	JA73	-0.32	0.00		SJ28	1.64E-08	SA27	SA40	-5.01	0.00
JJ26	5.36E-13	JA30	JA71	-3.66	0.00		SJ29	1.37E-11	SA33	SA41	-9.60	0.00
JJ27	3.72E-10	JA12	JA79	-0.32	0.00		SJ30	3.96E-12	SA17	SA49	-6.53	0.00
JJ28	8.22E-08	JA64	JA82	-2.52	0.00		SJ31	4.12E-08	SA17	SA34	11.48	7.76
JJ29	9.31E-08	JA32	JA46	-0.33	0.00		SJ34	3.48E-11	SA18	SA35	-11.58	0.00
JJ30	7.91E-08	JA21	JA72	-0.26	0.00		SJ35	6.56E-12	SA1	SA49	-9.21	0.00
JJ30	7.91E-08	JA30	JA72	-0.26	0.00		SJ36	5.60E-08	SA30	SA52	3.50	0.49
JJ31	7.40E-11	JA45	JA50	2.55	0.92	-	SJ37	1.77E-11	SA36	SA37	12.17	2.66
JJ32	8.27E-13	JA64	JA80	-7.49	0.00		SJ38	1.43E-10	SA26	SA30	-8.09	0.00
JJ33	1.87E-10	JA33	JA50	-2.87	0.00		SJ39	1.87E-11	SA13	SA15	-10.58	0.00
JJ34	3.25E-09	JA42	JA75	2.00	2.00	-	SJ40	1.06E-12	SA24	SA26	-8.09	0.00
JJ35	1.01E-12	JA14	JA18	-7.92	0.00		SJ41	8.13E-16	SA21	SA41	-13.23	0.00
JJ36	8.47E-11	JA71	JA73	-5.77	0.00		SJ42	8.46E-12	SA46	SA49	-2.81	0.00
JJ37	1.47E-09	JA8	JA22	-1.20	0.00		SJ43	8.78E-12	SA22	SA23	-3.89	0.00
JJ38	2.02E-09	JA28	JA60	-5.89	0.00		SJ44	6.96E-11	SA9	SA22	-10.01	0.00
JJ39	7.62E-15	JA56	JA65	-13.72	0.00		SJ45	9.39E-11	SA30	SA53	-10.07	0.00
JJ40	3.82E-11	JA64	JA71	4.24	4.00	+	SJ46	1.07E-11	SA33	SA37	0.43	0.43
JJ41	8.71E-08	JA23	JA71	1.69	0.02	-	SJ47	9.76E-15	SA33	SA49	-2.75	0.00
JJ42	4.50E-08	JA8	JA63	-0.78	0.00		SJ48	2.27E-09	SA49	SA52	-0.34	0.00
JJ43	7.01E-10	JA43	JA57	-3.72	0.00		SJ49	1.79E-07	SA23	SA34	11.23	4.36

JJ44	2.13E-09	JA10	JA62	2.38	1.17	-	SJ50	6.49E-10	SA30	SA34	8.58	8.58	*
JJ45	1.91E-06	JA5	JA44	1.21	0.05	-	SJ51	9.07E-12	SA32	SA39	19.14	13.46	*
JJ46	4.71E-08	JA37	JA40	-3.56	0.00		SJ53	1.84E-11	SA10	SA34	-5.57	0.00	
JJ47	3.87E-08	JA39	JA42	0.59	0.01	-	SJ54	2.86E-10	SA40	SA43	12.76	5.27	*
JJ48	3.81E-10	JA57	JA77	-6.56	0.00		SJ56	1.60E-11	SA27	SA36	6.73	5.57	*
JJ49	3.13E-13	JA72	JA80	-12.40	0.00		SJ57	1.18E-09	SA21	SA26	-3.28	0.00	
JJ50	1.08E-08	JA19	JA41	1.21	0.79	-	SJ59	2.05E-10	SA2	SA22	-3.99	0.00	
JJ51	3.16E-09	JA10	JA45	-7.87	0.00		SJ60	1.21E-10	SA13	SA44	-10.34	0.00	
JJ52	2.96E-14	JA5	JA74	-14.30	0.00		SJ61	6.53E-11	SA32	SA36	-1.79	0.00	
JJ53	1.45E-14	JA10	JA18	-14.95	0.00		SJ62	2.24E-11	SA2	SA50	4.77	4.77	*
JJ54	4.95E-15	JA18	JA74	-15.49	0.00		SJ63	3.89E-11	SA11	SA19	-3.07	0.00	
JJ55	6.47E-10	JA38	JA39	-0.52	0.00		SJ64	8.20E-08	SA5	SA30	-8.32	0.00	
JJ56	5.64E-12	JA71	JA74	-6.04	0.00		SJ65	1.66E-11	SA22	SA45	-3.21	0.00	
JJ57	2.81E-12	JA14	JA36	-12.42	0.00		SJ66	3.98E-10	SA27	SA45	1.83	1.83	+
JJ58	3.22E-14	JA19	JA37	-10.67	0.00		SJ67	7.63E-16	SA4	SA46	-9.75	0.00	
JJ59	1.03E-13	JA5	JA32	-14.15	0.00		SJ68	8.16E-11	SA34	SA45	-3.14	0.00	
JJ60	7.98E-09	JA54	JA60	6.45	1.23	-	SJ69	2.64E-09	SA17	SA45	-5.71	0.00	
JJ61	4.69E-09	JA33	JA60	6.59	4.05	+	SJ70	7.89E-12	SA16	SA26	-11.34	0.00	
JJ62	6.29E-12	JA59	JA60	15.61	8.54	*	SJ71	9.34E-14	SA4	SA46	-6.44	0.00	
JJ63	1.53E-07	JA24	JA72	6.87	1.10	-	SJ72	3.18E-11	SA30	SA45	0.71	0.71	+
JJ64	2.49E-07	JA39	JA47	2.07	1.25	-	SJ73	4.25E-10	SA10	SA34	-5.42	0.00	
JJ65	1.83E-08	JA39	JA47	-2.04	0.00		SJ74	1.48E-11	SA4	SA43	-0.64	0.00	
JJ66	1.87E-09	JA19	JA72	-4.57	0.00		SJ75	8.98E-13	SA26	SA30	-14.51	0.00	

