

Towards breeding for resistance against currant-lettuce aphid



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The currant-lettuce aphid *Nasonovia ribisnigri* is the most significant pest infesting lettuce in northern Europe. Its presence at harvest makes heads and salad packs unmarketable with significant financial losses for growers. Until recently, the most effective control method for *N. ribisnigri* was the use of resistant cultivars of lettuce. These resistant cultivars are grown widely, but the selection pressure induced by the reliance on a single resistance gene has inevitably now resulted in a new currant-lettuce aphid biotype that is able to thrive on 'resistant' plants possessing the *Nr* resistance gene. The identification of new mechanisms of resistance is therefore required urgently.

Genebanks holding diverse lettuce germplasm offer a potential source of beneficial alleles that may confer resistance.

Large genebank collections can be difficult and costly to screen for many agronomically important traits.

We have assembled a diversity set (DS) of 96 lettuce and wild species lines that is amenable to routine phenotypic analysis (Figure 1).



Figure 1. Example of morphological variation in the lettuce DS. Lines a-h represent different *L. sativa* crop types. Lines i-k represent examples of the wild *Lactuca* species.

Transcriptome sequence reads from two lettuce cultivars were aligned with lettuce ESTs to identify SNPs. SNPs were converted into KASPar assays, and used to genotype the diversity set.

Genotype data were used to explore phylogenetic relationships in the lettuce DS (Figure 2).

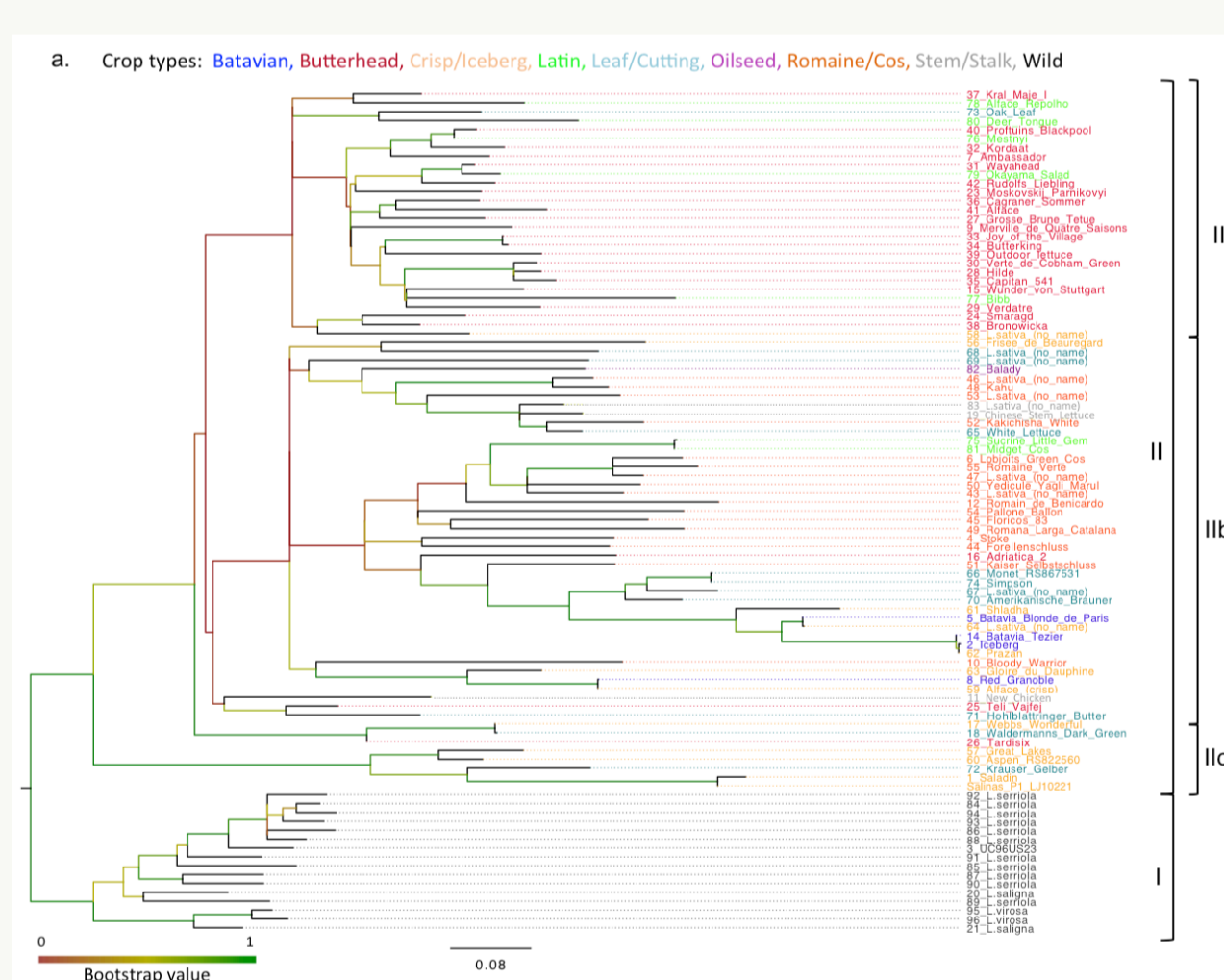
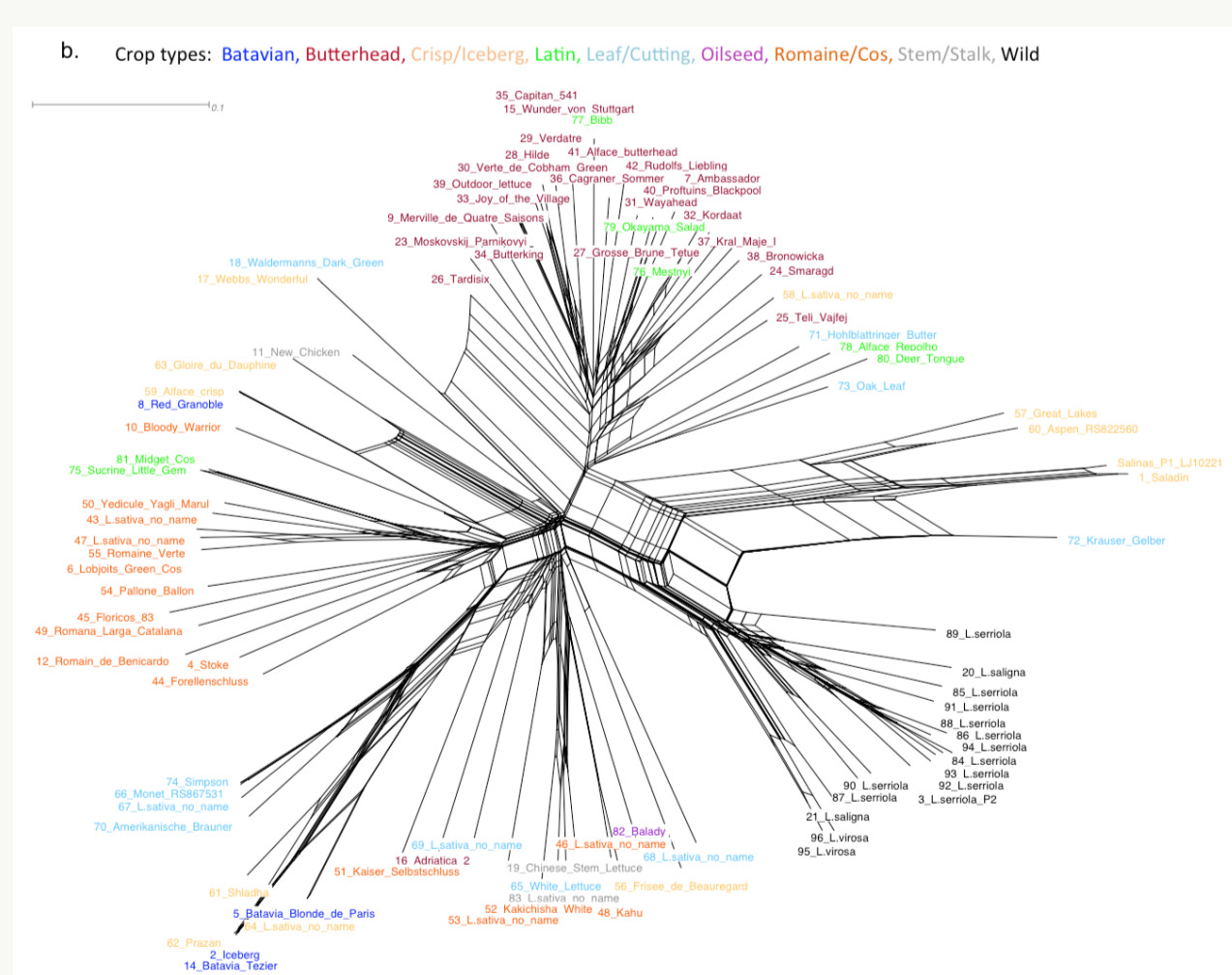


Figure 2. Genetic relationships in the VeGIN lettuce DS collection.

a. Dendrogram of relationships between the 96 lettuce DS lines. Cluster-I wild lettuce species, cluster-II domesticated species, sub-divided into cluster-IIa, encompassing Butterhead-like types, cluster-IIb encompassing Romaine/Cos types, and cluster IIc representing an evolutionarily distinct clade.



b. Neighbour-Net splits graph showing inferred evolutionary relationships and likely recombination events among accessions.

Genotype data were used to quantify population sub-structure in the diversity set (Figure 3).

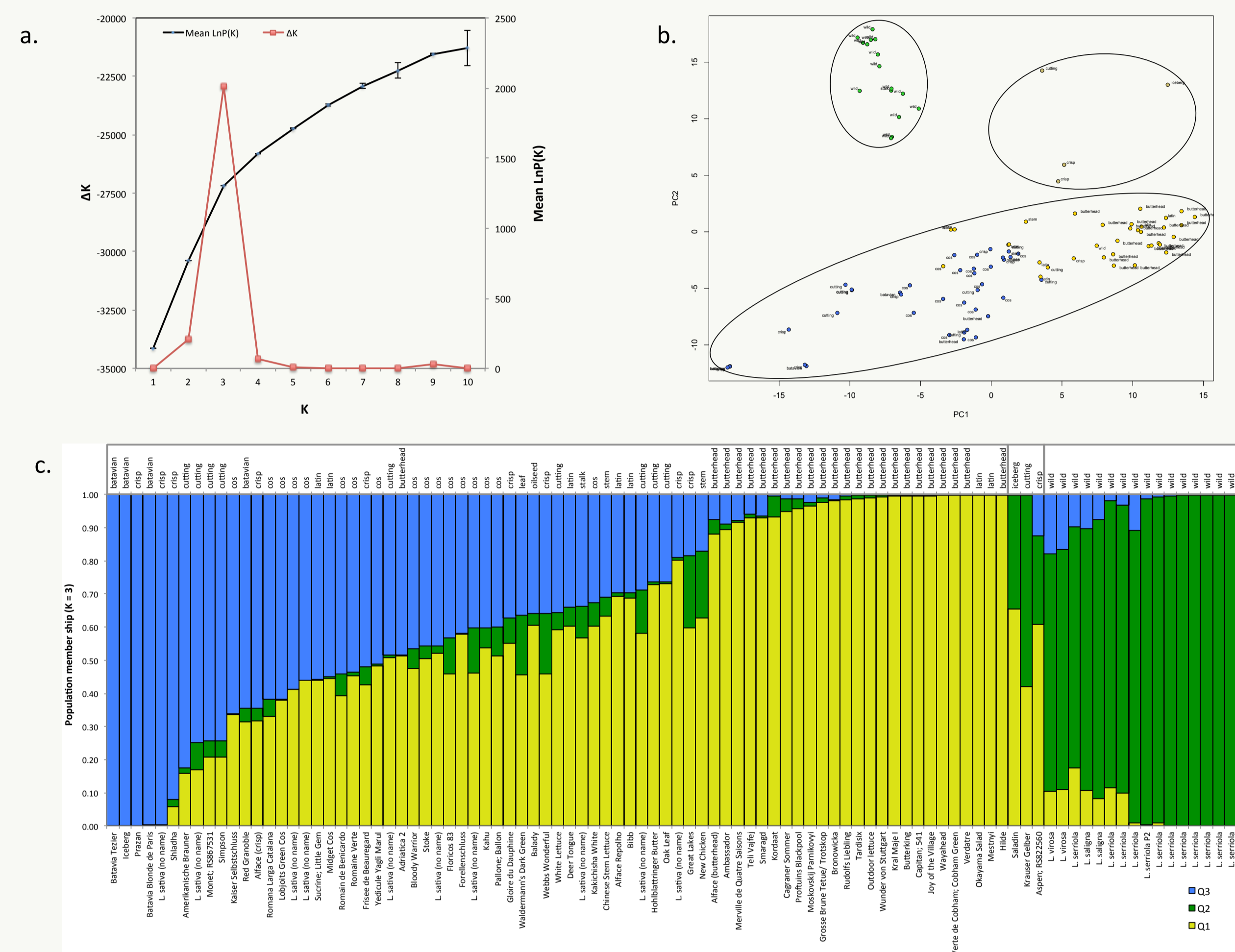


Figure 3. Population structure in the Lettuce DS. a. ΔK and mean $\text{LnP}(K)$ values derived from STRUCTURE output, $K = 3$ was chosen as the optimal K value; b. Plot of the first two eigenvectors that explained the most genotypic variation during principal components analysis: green = wild species, salmon = distinct sub-clade, the remaining domesticates are divided into yellow = Butterhead-like, blue = remaining accessions including predominantly Cos/ Romaine types; c. Bar plot of sub-population membership when $K = 3$ (Q1, Q2, Q3).

To quantify aphid resistance we inoculated lines from the lettuce DS with *N. ribisnigri* and recorded the number of alates (winged), apterae (non-winged), and the total number of aphids (Figure 4).

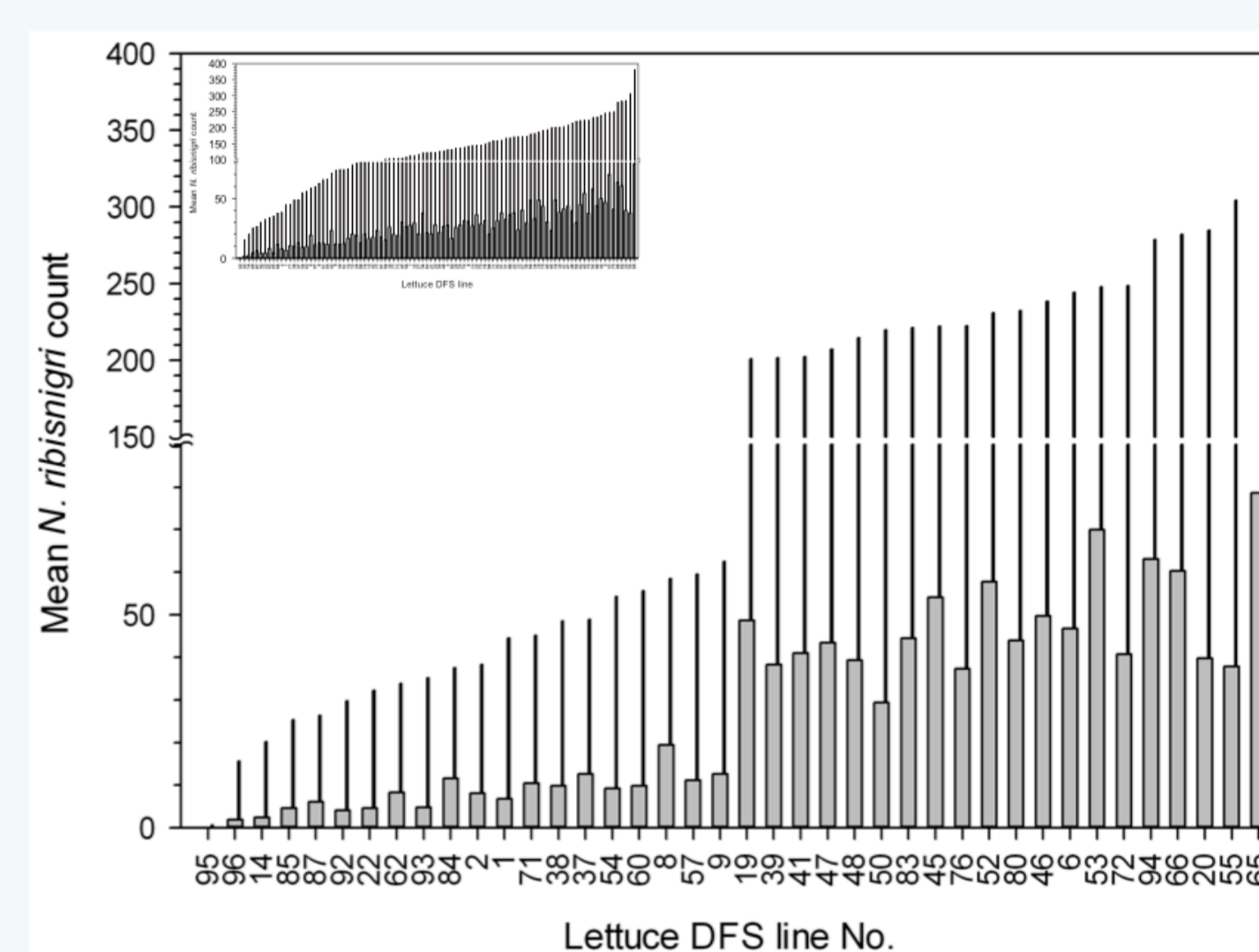


Figure 4. Mean numbers of *N. ribisnigri* recorded three weeks post-inoculation on the 96 lettuce DS. Mean numbers of alate (grey bar), and total number of *N. ribisnigri* (black needle) are presented. For clarity, the 40 lines illustrated are taken from the extremes of the total distribution (insert).

Phenotype and genotype data were used in combination with 'q' and kinship matrices during association analyses (Figure 5).

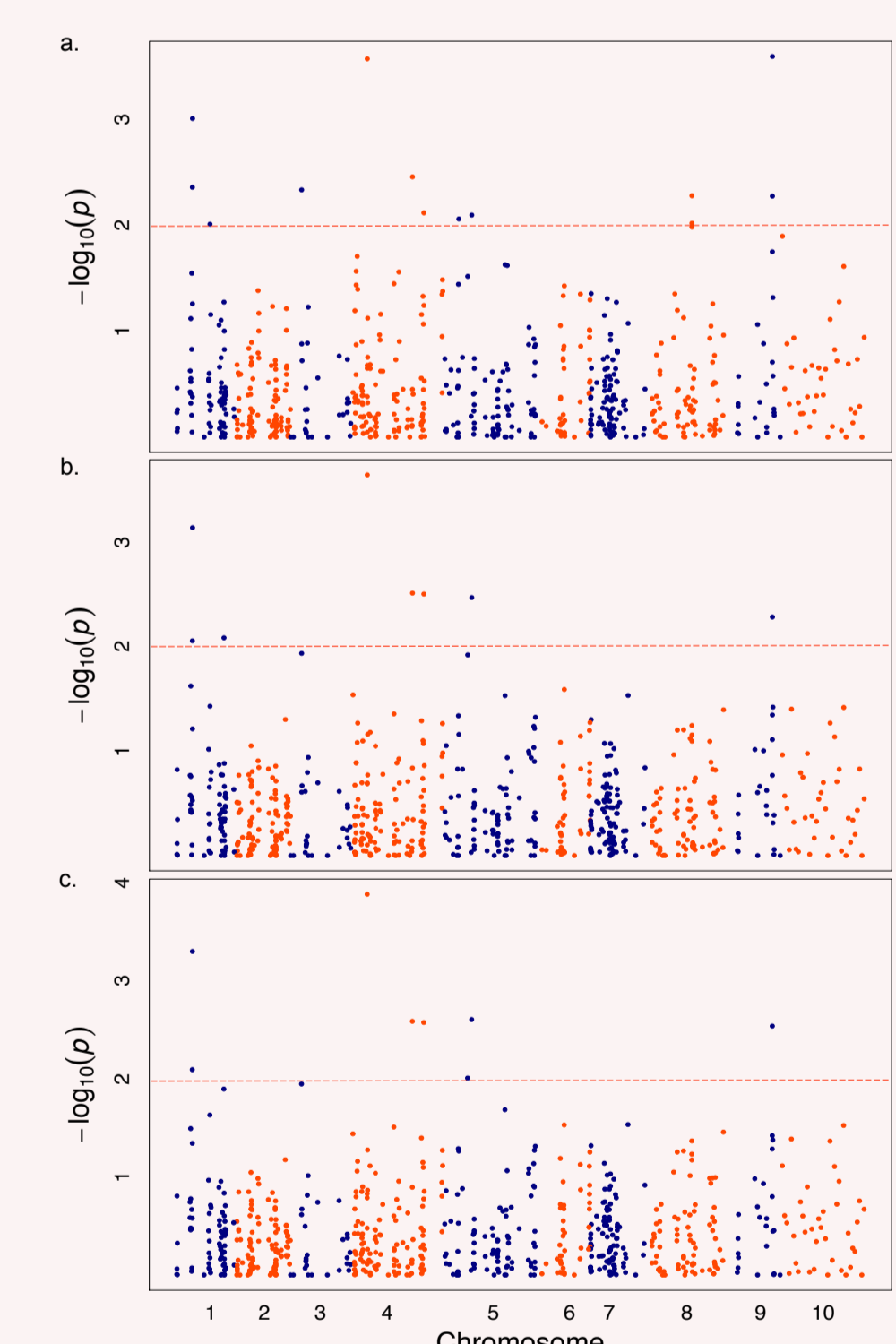


Figure 5. *L. sativa* genome wide distribution of $-\log_{10} P$ values of SNP associations with *N. ribisnigri* count data. Panels' a., b., and c. represent SNP associations with mean count data for alates, apterae, and total aphid count respectively. Chromosome 10 = unmapped SNPs.

- The identification of SNPs that have a significant association with resistance against aphid morph types presents the opportunity to use these as markers during selection of progeny in the breeding of new cultivars. These new cultivars may offer an additional line of defence when combined in an integrated pest management system.
- We are now attempting to create an aphid mapping population, with aphid specific markers to explore plant-insect interaction networks.