Genetic architecture of ecological divergence between two wild rice species (Oryza rufipogon and O. nivara)

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Abstract

Ecological divergence due to habitat difference plays a prominent role in the formation of new species but the genetic architecture during ecological speciation and the mechanism underlying phenotypic divergence remain less understood. Two wild rice species (O. rufipogon and O. nivara) are a progenitor-derivative species pair with ecological divergence and provide a unique system for studying ecological adaptation/speciation. Here, we constructed a high-resolved linkage map and conducted a quantitative trait locus (QTL) analysis of 19 phenotypic traits using an F2 population generated from a cross between the two wild rice species. We identified 113 QTLs associated with interspecific divergence of 16 quantitative traits, with effect sizes ranging from 1.61% to 34.1% in terms of the percentage of variation explained (PVE). The distribution of effect sizes of QTLs followed a negative exponential, suggesting that a few genes of large effect and many genes of small effect were responsible for the phenotypic divergence. We observed 18 clusters of QTLs (QTL hotspots) on 11 chromosomes, significantly more than that expected by chance, demonstrating the importance of coinheritance of loci/genes in ecological adaptation/speciation. Analysis of effect direction and v-test statistics revealed that interspecific differentiation of most traits was driven by divergent natural selection, supporting the argument that ecological adaptation/speciation would proceed rapidly under coordinated selection on multiple traits. Our findings provide new insights into the understanding of genetic architecture of ecological adaptation and speciation in plants and helps effective manipulation of specific genes or gene cluster in rice breeding.

Research Article

Genetic architecture of ecological divergence between two wild rice species (*Oryza rufipogon* and *O. nivara*)

Running title: Genetic architecture of two divergent wild rice species

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Abstract

Ecological divergence due to habitat difference plays a prominent role in the formation of new species but the genetic architecture during ecological speciation and the mechanism underlying phenotypic divergence remain less understood. Two wild rice species (*O. rufipogon* and*O. nivara*) are a progenitor-derivative species pair with ecological divergence and provide a unique system for studying ecological adaptation/speciation. Here, we constructed a high-resolved linkage map and conducted a quantitative trait locus (QTL) analysis of 19 phenotypic traits using an F_2 population generated from a cross between the two wild rice species. We identified 113 QTLs associated with interspecific divergence of 16 quantitative traits, with effect sizes ranging from 1.61% to 34.1% in terms of the percentage of variation explained (PVE). The distribution of effect sizes of QTLs followed a negative exponential, suggesting that a few genes of large effect and many genes of small effect were responsible for the phenotypic divergence. We observed 18 clusters of QTLs (QTL hotspots) on 11 chromosomes, significantly more than that expected by chance, demonstrating the importance of coinheritance of loci/genes in ecological adaptation/speciation. Analysis of effect direction and$v$ -test statistics revealed that interspecific differentiation of most traits was driven by divergent natural selection, supporting the argument that ecological adaptation/speciation would proceed rapidly under coordinated selection on multiple traits. Our findings provide new insights into the understanding of genetic architecture of ecological adaptation and speciation in plants and helps effective manipulation of specific genes or gene cluster in rice breeding.

Key words: Genetic architecture, QTL mapping, ecological speciation, phenotypic traits, wild rice

Introduction

Ecological divergence between populations, often arising from local adaptation, is driven by divergent natural selection between contrasting environments, which in turn results in ecological speciation through the evolution of reproductive isolation (Nosil, 2012; Seehausen *et al* ., 2014; Schluter & Rieseberg, 2022). Multiple lines of evidence from plant and animal studies have demonstrated that ecological divergence due to habitat difference plays a prominent role in the formation of new species (Erickson *et al* ., 2004; Peichel & Marques, 2017; Schluter & Rieseberg, 2022). During the process of ecological speciation, phenotypic differentiation occurs and reproductive isolation evolves as a consequence of divergent natural selection (Erickson *et al* ., 2004; Nosil, 2012; Faria *et al* ., 2014; Schluter & Rieseberg, 2022). Despite substantial studies, many fundamental questions regarding the genetic basis underlying ecological divergence and speciation remain debated or largely elusive, including the relative contributions of loci/genes with large vs. small effects to phenotypic divergence, the randomly distributed vs. clustered genetic architecture and their evolutionary implications, mechanisms underlying the link between evolution of divergent phenotypes and the emergence of reproductive isolation, and the role of divergent selection in the process of ecological divergence and speciation (Faria *et al.,*2014; Seehausen *et al* ., 2014; Nosil *et al* ., 2021; Kitano*et al* ., 2022; Bomblies & Peichel, 2022; Schluter & Rieseberg, 2022).

Two wild rice species, *O. rufipogon* Griff. and *O. nivara*Sharma et Shastry, are most closely related and collectively regarded as the progenitors of cultivated rice (*O. sativa* L.) (Khush, 1997; Sang & Ge, 2007; Vaughan *et al* ., 2008; Cai *et al* ., 2019). The perennial *O. rufipogon* , characterized by photoperiod sensitivity and predominate cross-fertilization, is widely distributed throughout southern China, South and Southeast Asia, Papua New Guinea and northern Australia. In contrast, the annual *O. nivara* , characterized by photoperiod insensitivity and predominant self-fertilization, has a more restricted distribution in South and Southeast Asia (Vaughan, 1994; Sang & Ge, 2007; Vaughan *et al* ., 2008). In addition, interspecific differences in a few dozens of traits have been documented by experimental and field investigations (Sano*et*

al ., 1980; Morishima *et al* ., 1984; Barbier, 1989; Banaticla-Hilario *et al* ., 2013; Guo *et al* ., 2016; Cai *et al* ., 2019; Ren, 2019; Eizenga *et al* ., 2022; Jing *et al* ., 2023). Moreover, studies show that the annual *O. nivara* evolved from the perennial*O. rufipogon* to associate with a habitat shift from a persistently wet to a seasonally dry habitat, in which flowering time change in the derived *O. nivara* was the major component contributing to the reproductive isolation between *O. rufipogon*and *O. nivara* (Morishima *et al* ., 1984; Barbier, 1989; Cai*et al* ., 2019; Xu *et al* ., 2020). Therefore, the progenitor-derivative species pair of wild rice with distinct differences in morphology, life history traits and habitat preference, represents a feasible system for the study of ecological adaptation and speciation (Grillo *et al* ., 2009; Zheng & Ge, 2010; Cai *et al* ., 2019).

QTL analysis is powerful approach to uncover the genetic architecture of ecologically important traits and to determine the targets of natural selection and thus has been used successfully for studies of evolutionary process and mechanisms in various plants and animals (Tanksley, 1993; Barton & Keightley, 2002; Erickson *et al* ., 2004; Saltz *et al* ., 2017; Jakobson & Jarosz, 2020; Connallon & Hodgins, 2021). In this study, we present a quantitative traits locus (QTL) analysis of an F_2 population derived from a cross between *O. rufipogon* and *O. nivara* , using SNPs generated from specific-locus amplified fragment sequencing (SLAF-seq) technology (Sun *et al.,* 2013). First, we examine the number, effect size and distribution pattern of QTLs controlling phenotypic divergence between species. Specifically, we ask: 1) How many genomic regions contribute to the phenotypic divergence between *O. rufipogon* and *O. nivara* given substantial phenotypic differentiation between species? 2) Are the traits differentiating two species controlled by a large number of loci with small effects or a small number of loci with large effects? Although substantial studies involving morphological variation have been undertaken on *O. nivara* and *O. rufipogon* (e.g., Morishima*et al* ., 1961; Barbier, 1989; Cai *et al* ., 2004; Banaticla-Hilario *et al* ., 2013; Guo *et al* ., 2016; Kim*et al* ., 2016; Cai *et al* ., 2019; Eizenga *et al* ., 2022), no effort has been attempted to explore the genetic basis of phenotypic divergence between the two species until Grillo *et al* . (2009) who performed a QTL analysis to investigate the genetic architecture for phenotypic divergence between *O. rufipogon* and*O. nivara* . Nevertheless, Grillo *et al* . (2009)'s study provided limited knowledge of the genetic basis underlying phenotypic divergence because of the low marker density (116 SSRs) and relatively small mapping populations (less than 200). Here, based on the high-resolution markers, we were able to identify QTLs for phenotypic traits that diverge between species and explore the full genetic architecture of ecological speciation.

Second, we address how the identified loci are distributed across the genome and whether they cluster (colocalize) in particular chromosomal regions. Multiple lines of evidence indicate that adaptation to multiple different aspects of new environments can be facilitated by coinheritance of adaptive phenotypes, embodied as enriched QTLs in some genomic regions (Hoffmann & Rieseberg, 2008; Jacobs *et al* ., 2017; Nosil *et al* ., 2021; Bomblies & Peichel, 2022). Indeed, accumulating studies in both plants (e.g., Nakazato *et al* ., 2013; Lowry *et al* ., 2015; Ferris *et al* ., 2017; Roda *et al* ., 2017) and animals (e.g., Linnen *et al* ., 2013; Jacobs*et al* ., 2017; Archambeault *et al* ., 2020) revealed that many QTLs were not distributed randomly across the genome but rather in hotspots involving a variety of adaptive traits. Clustering of QTLs responsible for domestication-related traits is also common in crop species (e.g., Burke *et al* ., 2002; Cai & Morishima 2002; Wang*et al* ., 2011; Yang *et al* ., 2019; Geng *et al* ., 2021). These studies suggested that QTL clustering, due to either pleiotropy or tight linkage, might be a mechanism for preventing unfit combinations of genotypes and thus facilitates rapid adaptation and speciation (Peichel & Marques, 2017; Nosil *et al* ., 2021; Bomblies & Peichel, 2022). Despite these, the prevalence of the clustered genetic architecture and the mechanisms that facilitate the coinheritance of adaptive phenotypes during ecological speciation are less understood (Yang *et al* ., 2019; Archambeault *et al* ., 2020; Bomblies & Peichel, 2022). The two *Oryza* species provide a unique opportunity to gain further insights into the distribution pattern of QTLs and the underlying mechanisms during ecological speciation.

Finally, we investigated the potential roles of natural selection in trait divergence between species. Evidence shows that the origin of*O. nivara* from *O. rufipogon* was associated with suite of phenotypic changes in a pattern consistent with ecological speciation (Morishima *et al* ., 1984; Barbier, 1989; Guo et al. 2016; Cai*et al* ., 2019; Ren 2019). Although previous studies demonstrated the roles of natural selection rather than

random genetic drift in the phenotypic divergence between *O. rufipogon* and *O. nivara*(Guo et al. 2016; Cai *et al.* , 2019), these studies were unable to distinguish between direct and indirect selections acting on the traits because selection on one trait could have caused substantial divergence in other traits due to genetic correlations (Via & Hawthorne, 2005; Muir *et al* ., 2014; Feng *et al* ., 2019). Recently developed$v$ test (Fraser 2020) provides a feasible and powerful approach to determine whether traits evolved under directional selection based on phenotype divergence of parental and phenotype distribution of the crossing population. Therefore, we were interested in whether divergent natural selection is responsible for the coordinated differentiation of a suite of traits as expected during ecological divergence between*O. rufipogon* and *O. nivara* . Addressing these questions not only provides additional insights into the process and mechanisms of ecological adaptation and speciation in plants but also facilitates rice genetic improvements given abundant unique genetic resources maintained in wild rice.

Materials and Methods

Development of F_2 mapping population

To explore the genetic basis of divergence between wild rice species, we created an F_2 mapping population between *O. rufipogon* (Ruf-I) and *O. nivara* (Niv-I) inbred lines (Fig. 1, Table S1) that were self-pollinated for five generations. The *O. rufipogon* and *O. nivara* individuals were sampled from India and showed morphologies typical of the two species that diverge significantly in numerous traits, including the taxonomically diagnostic characters such as flowering time, anther length, culm length, panicle exsertion and shape (Cai *et al* ., 2017; Jing *et al* ., 2023). The construction of inbred lines of two parents, crossing between parental lines and subsequent development of F_1 and F_2 populations were conducted from 2014 to 2017 at Lingshui Station (N18°30.6', E110°2.4') in Hainan Province, China (Meng, 2021).

Because our previous studies showed that *O. nivara* usually flowered over 60 days earlier than *O. rufipogon* in the wild (Cai*et al* ., 2019; Xu *et al* ., 2020), we germinated the seeds of*O. nivara* in three batches at an interval of 10 days to ensure the concurrence of the flowering time of the parental lines. The Niv-I was designated as female parent, while Ruf-I was selected as male parent. To avoid self-pollination in crossing, we emasculated the panicles of the female parents before blossoming of the spikelets and then removed the immature anthers, and finally sprayed water on the emasculated panicles in case of residual pollen (Xu *et al* ., 2020). A single F_1 individual from the crosses between Ruf-I and Niv-I was chosen randomly to produce the F_2population (N=1174) by selfing (Meng, 2021).

The F_2 population was grown together with self-fertilizing seeds from two mapping parents (Ruf-I, N = 28 and Niv-I, N = 23) in Lingshui Station in November, 2016. Seeds were processed at 50 °C for five days to break dormancy and then germinated in a growth chamber under long day condition (day: 14 h, 36 °C; night: 10 h, 33 °C). One week later, the young seedlings were planted in greenhouse at natural daylength condition. After additional 3 weeks, the seedlings with more than 3 tillers were transplanted into paddy field randomly with a spacing of 1.5 × 1.5 meters. Finally, 862 F_2 plants survived to the flowering period and were recorded phenotypically.

Phenotypic analyses

We measured 19 phenotypic traits (Table S2) that were either adaptive or taxonomically and agronomically important according to previous studies (Banaticla-Hilario *et al* ., 2013; Guo *et al* ., 2016; Cai*et al* ., 2019; Ren, 2019; Eizenga *et al* ., 2022; Jing*et al* ., 2023). Given premating reproductive isolation (flowering time difference) and habitat divergence between *O. rufipogon* and*O. nivara* are two main factors associated with the process of speciation, we classified all traits into three categories: reproduction-related traits (RR traits) (three quantitative traits), habitat-related traits (HR traits) (13 quantitative traits) and color traits (three qualitative traits) (Table S2). The RR traits was related to mating or reproductive isolation and the HR traits involved habitat preference of the derived *O. nivara* . Like many other studies on phenotypic variation in which different classifications of traits were used (e.g., Lexer *et al* ., 2005; Hall *et al* ., 2006; Grillo *et al* ., 2009; Peichel & Marques, 2017), trait division in our case seems a bit arbitrary but feasible to help our analyses by relating phenotypic variation to ecological speciation.

Trait measurements were taken on all plants that flowered following the methods detailed in Biodiversity-International *et al* . (2007). Measurements were taken for three tillers/culms and averaged for each trait, except for first heading, culm habit, grain and color traits (Table S2). First heading (FH) and culm habit (CH) were recorded for the primary culm. Grain length (GL) and width (GWI) were calculated for 10 full seeds and grain weight (GWE) were measured for 30full seeds. Three qualitative color traits that might be related with biotic and abiotic stress in plants (Qin *et al* ., 2021; Dabravolski *et al* ., 2023), i.e., awn color (AWC), basal leaf sheath color (BLSC), and stigma color (SC), were scored as binary traits, with 1 and 0 indicating the presence and absence, respectively (Table S2).

To test for trait divergence between parental populations, t -test and *chi-square* (χ^2) test were conducted for the 16 quantitative and three qualitative traits, respectively. We used the method of Pearson correlation to calculate the correlations among traits. All the calculations and plotting were performed in R (R Core Team, 2020).

Sequencing and genotyping

Fresh leaves of two parental lines and 600 F_2individuals randomly chosen from the 862 F_2 population were collected and dried by silica-gel. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (Murray & Thompson, 1980). The libraries of mapping parents were constructed following the manufacturer's recommendations (Illumina) for 500 bp insert size and sequenced by BGIseq500 platform (BGI; Shenzhen, China) with 150 bp paired-end reads. The F_2 individuals were genotyped by a specific-locus amplified fragment-sequencing (SALF-seq) method (Sun*et al.,* 2013). In brief, two restriction enzymes (*RsaI* and*HaeIII*) were selected to digest the genomic DNA. The digested fragments (SLAF tags) were ligated to the adapters with T4 DNA ligase. After PCR amplification, purification, sample mixing and electrophoresis with agarose gels, the size of fragments ranging from 264-314 bp were obtained and purified. Subsequently, the products were sequenced using Illumina HiSeq 2500 platform (Illumina; San Diego, U.S.) with 125 paired-end reads according to the manufacturer's instruction.

The short reads of parental lines and F_2 individuals were filtered by removing low-quality reads with more than 10% of bases missing. Then short reads were aligned to the reference sequence of Nipponbare genome (IRGSP-1.0) (Kawahara *et al.,* 2013) using BWA (Li & Durbin, 2010) with the MEM algorithm. Furthermore, Samtools (Li*et al* ., 2009) were applied to sort the mapping results and built index for each BAM file. Variant calling was conducted using the Genome Analysis Toolkit (GATK, version 4.0.2.1) (Van der Auwera *et al* ., 2013). SNPs were filtered with VariantFiltration of GATK "AC < 2 || QD < 2.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0" as suggested by the manual.

The filtered SNPs were genotyped based on the following criteria: (1) Homozygous for each mapping parent and different between two parents; (2) Bi-allelic polymorphism among the F_2 individuals; (3) The missing rate were no more than 5%; (4) The extreme segregation distorted SNPs were excluded at the cut-off (P [?] 1×10^7) in the separation of Mendelian law (Zhang*et al* ., 2010; Zuo *et al* ., 2019; Wang *et al* ., 2022). Finally, we obtained 38,144 SNPs in total.

Linkage map construction and QTL analyses

SNPs markers were converted into bins using SNPbinner (Gonda *et al* ., 2019; Oren *et al* ., 2019). Cross points were calculated with minimum ratio (r) set as 0.01 and bins were generated with minimum bin length (-m) set as 5 kb. Based on the position, 6,579 bin markers were divided into linkage groups corresponding 12 rice chromosomes (Fig. S1). We applied *est.rf* and *est.map* in R/qtl (Broman *et al* ., 2003) to demonstrate marker order and the genetic position of bins.

We performed QTL mapping in R/qtl (Broman *et al* ., 2003). The composite interval mapping (CIM) was conducted with *cim* function, the window size was set as 10 cM, and the cofactor was stepwise increased until the detected QTLs were stable. The genome wide significance threshold of each trait was determined separately by 1000 permutations with $\alpha = 0.05$. The epistasis between QTLs was detected by*addint* function, and QTL interaction pairs reached statistically significant ($P < 0.05$) were kept. We estimated the percent

variance explained (PVE), additive effect and dominance effect with *fitqtl* function. The confidence intervals were determined as 1.5-LOD confidence intervals.

The direction of QTL effect was scored as a positive if the effect of a parental allele was consistent with species divergence and otherwise, was scored as negative if the effect was opposite of species divergence. We noted that the threshold level for classification of QTLs in terms of effect size was variable in previous studies in which the criteria of 10% ˜ 25% of the total phenotypic variation have been used for a criterion of major QTLs (e.g., Tanksley *et al* ., 1993; Bradshaw *et al* ., 1998; Davey *et al* ., 2006; Hall *et al* ., 2006; Kumar *et al* ., 2017). For simplicity, we accept a criterion of 10% for distinguishing between major-effect and minor-effect QTLs and defined a major-effect QTL as a large-effect QTL if it explains $>$ 25% of the total phenotypic variance in the mapping population.

To explore the extent to which the QTLs overlapped across the genome and in their correlations with phenotypic traits, we first compared the distribution of the QTL peaks on each chromosome with uniform probability distribution using the Kolmogorov-Smirnov test (Massey, 1951), performed by using *ks.test* function in R. If the QTL positions significantly deviated from uniform distribution, the QTLs were not distributed randomly (Arnegard et al., 2014). Then, following the methods in previous studies (Nakazato *et al* ., 2013; Frary*et al* ., 2014; Oakley *et al* ., 2018), we marked all QTLs on the 12 chromosomes and identified the QTLs that exhibited overlapping 1.5-LOD confidence intervals with at least two other QTLs. Those regions with more than three QTLs overlapping across traits were defined as QTL hotspots (Nakazato *et al* ., 2013, Frary *et al* ., 2014).

Test for directional selection

To determine whether traits evolved under directional selection, we performed v test of Fraser (2020) based on phenotype divergence of parental and phenotype distribution of the crossing population. This method is a generalization of QTL sign test (Orr, 1998b) and applicable to phenotypic data for almost any genetic cross, thus providing a feasible and powerful approach to detect selection. The v test was performed as described in equation 2 of Fraser (2020). To calculate$v$, we estimated the phenotypic variances within and between parents of the cross, and the variance among F_2population. As the broad-sense heritability (H^2) was needed to correct the random noise, we calculated as V_g/V_p by using sommer package in R. The V_g is the genetic variance estimated from the kinship matrix and V_p is the phenotypic variance (Covarrubias-Pazaran, 2016). The constant c was equal to 2.0 for F_2 (Fraser, 2020). Significance of v was estimated based on the cumulative F distribution with (1,k -1) degree of freedom, the k is the individual number of F_2.

Results

Phenotypic variation and correlations

To evaluate variation patterns of phenotypic traits that diverged between *O. rufipogon* and *O. nivara* , we calculated the mean and ranges of 16 quantitative traits for two parental lines. The parental lines differed significantly for all traits (t -test,$P < 0.01$) except for spikelet number (SN) and culm diameter (CD) (Table 1). It is noted that all 14 traits that diverged significantly between species except for two flag leaf traits (FLL and FLW) exhibited the same differentiation patterns to those reported in previous studies using population samples (Banaticla-Hilario *et al* ., 2013; Guo *et al* ., 2016; Cai *et al* ., 2019; Ren, 2019). Consistent with previous argument (Guo *et al* ., 2016; Cai*et al* ., 2019), we regarded these 12 traits as adaptive traits (Table 1). Of three qualitative (color) traits, two (BLSC and SC) exhibited significant interspecific divergence (Table 1). However, divergent patterns for these color traits might represent the variation within populations/species because no significant differentiation was found between species for them in previous studies of natural populations (Cai *et al* ., 2019; Ren, 2019).

Overall, all 16 quantitative traits, including the two (SN and CD) without significant divergence between two parental lines, showed an increase in variance in F_2 population relative to the parental lines (Fig. 2), suggesting the segregation of many genes of small to moderate effect. It is evident that most traits were distributed normally or nearly normally (Fig. 2, Table S3), further suggesting that they are under polygenic

control. Three exceptions include two panicle traits (PE and PS) that exhibited largely bimodal distribution and the first heading (FH) that showed an extended tail in one direction, implying that these traits may be under the control of genetic loci with major genetic effects. Seven traits (ANL, CD, CH, CL, PL, SN, and FLL) showed obvious transgressive segregation in the F_2 population (Fig. 2).

We calculated the pairwise correlation of traits in F_2population to evaluate the potential roles of single pleiotropic or tightly linked loci in trait divergence between species because a significant correlation between traits suggests the shared genetic basis due to either pleiotropy or linkage of genes (Via & Hawthorne, 2005; Saltz *et al* ., 2017). As shown in Fig. 3, 75 (63%) of all 120 pairwise combinations of 16 quantitative traits showed significant correlations, with most of them (89%) being positive. By focusing analyses on 12 putatively adaptive traits, we detected significant correlations for two pairwise combinations of three RR traits (ANL, FH, and PE) and for 20 (55.6%) of all 36 pairwise combinations of nine HR traits, with four traits (ANL, CL, PL, and PS) significantly correlating with almost all other traits (Fig. 3). These observations provided an initial indication that single pleiotropic or multiple tightly linked loci may have substantial impacts on the trait divergence between*O. rufipogon* and *O. nivara* .

Linkage map and QTL analysis

Based on 6579 bin markers, we constructed the genetic map spanning 1481.09 cM over 12 LGs corresponding 12 rice chromosomes, with the length close to cultivated rice (Huang *et al* ., 2009). The genetic distance between adjacent bin markers ranged from 0.08 cM to 4.62 cM, with the mean distance being 0.23 cM (Fig. 4, Table S4). The genetic map was high-resolution and enables us to get a comprehensive and precise mapping result.

To reveal the genetic basis of species divergence between *O. rufipogon* and *O. nivara* , we mapped QTLs involved in all 19 traits. For 16 quantitative traits, we identified a total of 113 QTLs that were located on all 12 chromosomes, with the number of QTLs per trait ranging from 4 (GWE and GWI) to 11 (ANL), and the amount of variation explained by these QTLs ranging from 1.61% (ANL3.b) to 34.1% (AWL4) (Table 2, Table S5). Moreover, we identified 12 major QTLs, i.e., the QTLs that explain over 10% of total phenotypic variation, which involved 10 traits (Fig. 4, Table 2). Of 12 major QTLs, three (AWL4, FLW1 and SN1.a) exhibit large effect, i.e., the QTLs that explain over 25% of total phenotypic variation. For three qualitative (color) traits, we identified five QTLs (one each for AWC and BLSC, and three for SC) (Table S5).

We detected significant epistatic interactions for 23 pairs of QTLs that affected 11 quantitative traits, with the number ranging from one pair (CL, FLW, GWI, and PS) to five (AWL) (Table 3). Of these combinations, eight involved major-effect QTLs (PVE $>$ 10%). However, all the significant epistatic interactions explained a small amount of variance, implying that the QTL $\times$ QTL interactions might not play an important role in divergence of these traits between species (Nakazato*et al* ., 2013).

Analysis of directional selection

Of 12 putatively adaptive traits, seven (ANL, FH, AWL, GL, GWI, PL, and PS) included at least one major-effect QTL (Table 2). Moreover, the majority of QTLs of all putatively adaptive traits except for three (GL, PL, and PS) were positive, with overall 77% (46/60) QTLs showing effects in the same directions of the phenotypic divergence between species (positive effect direction). These results suggested that differentiations of these putatively adaptive traits might be due to directional selection. The three exceptional traits included almost half of QTLs with negative effect direction (i.e., antagonistic effects) (Table 2), implying that these traits diverged under either weak selection or drift (Muir *et al* ., 2014; Ferris *et al* ., 2017).

To further explore the role of natural selection in trait divergence between *O. nivara* and *O. rufipogon* , we conducted *v*test for 12 putatively adaptive traits and found that eight traits were significant, including three RR traits, i.e., first heading (FH), anther length (ANL) and panicle exsertion (PE), which associate with reproductive isolation between species (Table 2). These results were in accordance with the above QTL effect analyses in which an overall proportion of positive QTLs was 77% across 12 adaptive traits tested, a reflection of interspecific differentiation of these adaptive traits under directional selection.

Distribution of effect sizes and clustering of QTLs

To evaluate the relative contribution of the mutations of large and small effects during phenotypic differentiation between the two species, we estimated the distribution of effect sizes for all 113 QTLs of 16 quantitative traits identified in the F_2 population (Fig. 5a). It is clear that the effect sizes of these QTLs were typically small to moderate ($< 10\%$ of PVE), with only three being the large-effect QTLs ($> 25\%$ of PVE), which is consistent with the Orr's model (Orr, 1998a) in which a few genes of large effect and many genes of small effect underlying the phenotypic divergence. The distribution of effect size for each of two categories (RR and HR traits as well as putatively adaptive traits) (Figs. 5b to 5d) also followed the Orr's model. These results suggest that the phenotypic evolution during the origin of *O. nivara* involves a dozen of traits through a few mutations of large effect and many mutations of small effect.

We first tested whether the QTL positions significantly deviated from uniform distribution on chromosomes using the Kolmogorov-Smirnov test (Massey, 1951; Arnegard et al., 2014) and found that QTLs on chromosomes 1, 4, 5, 6, 9 and 12 were significantly clustered rather than over-dispersed (Table S6). Then, by marking all QTLs on 12 chromosomes, we identified a total of 18 QTL hotspots located on 11 chromosomes, each involved traits from 3 to 13 (Fig. 4, Table S7). Interestingly, all major-effect QTLs (PVE $> 10\%$) except for one (GL1.b) were located in QTL hotspots, with four in the hotspots on chromosome 1 (FLW1, GWI1, PL1 and SN1.a) and chromosome 6 (FH6, FLL6, PL6.a, and PS6.a), one each in hotspots on chromosomes 3 (FH3), 4 (AWL4) and 5 (ANL5.b) (Fig. 4, Table S7).

It is noteworthy that a hotspot on chromosome 1 (HS1) involved nine species-distinguishing traits, including three RR traits (ANL, FH, and PE) and six HR traits (AWL, GL, GWE, GWI, PL, and PS) (Fig. 4, Table S7). Similarly, a hotspot on chromosome 6 (HS11) involved eight putatively adaptive traits (ANL, FH, PE, AWL, CH, CL, PL, and PS). Other hotspots all included the QTLs involving adaptive traits (Fig. 4, Table S7). Moreover, significantly positive correlations were detected for pairwise combinations of most adaptive traits with QTLs in the hotspots (Fig. 3). For example, in the hotspot on chromosome 6 (HS11) where eight adaptive traits were involved, panicle shape (PS) was significantly correlated with all other adaptive traits, and similarly, anther length (ANL) was significantly correlated with all other adaptive traits except for culm habit (CH) (Fig. 3). These results implied that a shared genetic basis (pleiotropy or linkage of multiple genes) might contribute to clustering of the QTLs responsible for interspecific trait divergence.

Discussion

Phenotypic divergence between *O. nivara* and *O. rufipogon* and the underlying genetic architecture

Phenotypic variation within and between *O. rufipogon* and *O. nivara* have been extensively investigated (e.g., Morishima *et al* ., 1961, 1984; Barbier, 1989; Vaughan, 1994; Cai *et al* ., 2004; Banaticla-Hilario *et al* ., 2013; Cai *et al* ., 2019; Ren, 2019; Eizenga *et al* ., 2022) because these two species are direct progenitors of cultivated rice with abundant genetic variation. Our recent studies incorporating common garden experiment, artificial crossing, and population genomics (Guo *et al* ., 2016; Cai *et al* ., 2019; Ren, 2019; Xu *et al* ., 2020) further demonstrated that the significant differentiation between species for a dozen of phenotypic traits were associated with habitat differences, as expected for ecological speciation (Zheng & Ge, 2010; Cai *et al* ., 2019; Ren, 2019). In the present study, we showed that most of the phenotypic traits divergent between species exhibited approximately normal distribution, i.e., quantitative or polygenic traits. Of 16 quantitative traits examined, 12 showed significant differentiation as expected for ecological divergence between *O. rufipogon* and *O. nivara*(Table 2) and these traits associated either with reproductive isolation between species or with the fitness of *O. nivara* in dry habitats (Grillo *et al* ., 2009; Banaticla-Hilario *et al* ., 2013; Cai*et al* ., 2019; Xu *et al* ., 2020). Notably, considerable interspecific divergence (> 1.5-fold differences) was observed for several traits commonly used for distinguishing species (i.e., diagnostic traits), including anther length (ANL), first heading (FH), panicle exsertion (PE), culm length (CD), and panicle shape (PS) (Banaticla-Hilario *et al* ., 2013; Cai *et al* ., 2019; Ren 2019; Eizenga *et al* ., 2022; Jing *et al* ., 2023). Overall, compared with the perennial *O. rufipogon* , the annual *O. nivara* flowers earlier with shorter anthers, is shorter with erected

flag leaves, has shorter and less exserted panicles with more compactness, and shorter culm length (plant height), consistent with previous studies (Grillo *et al* ., 2009; Banaticla-Hilario *et al* ., 2013; Guo *et al* ., 2016; Cai *et al* ., 2019; Eizenga*et al* ., 2022; Jing *et al* ., 2023).

Relative to numerous studies that investigated genetic basis of trait divergence between cultivated rice and either *O. rufipogon* or*O. nivara* (e. g., Xiong *et al* ., 1999; Thomson *et al* ., 2003; Uga *et al* ., 2003; Onishi *et al* ., 2007; Wang*et al* ., 2011; Luo *et al* ., 2016), only a single study (Grillo *et al* ., 2009) was performed to explore on the genetic architecture of divergence between *O. rufipogon* and *O. nivara* . Grillo *et al* . (2009) identified a total of 30 QTLs involving eight quantitative traits related to life history, mating system, and flowering time and found that the effect sizes of QTLs ranged from 2.9% to 36.5% with an exponential distribution. Nevertheless, the low marker density and small mapping population in Grillo *et al* . (2009)'s study would result in low mapping resolution and overestimate the percent variance explained for small-effect loci (Visscher *et al* ., 1996; Slate, 2005; Wang*et al* ., 2011; Connallon & Hodgins, 2021), which precludes further investigations on genetic basis of phenotypic divergence between the two species in depth.

In the present study, using a larger mapping population and higher marker density, we identified a higher number of QTLs (119) responsible for 19 phenotypic traits with much narrower regions (Fig. 4, Table S5). We noted that only 7 of 30 QTLs identified in Grillo *et al* . (2009) were re-located in our study. The differences might arise from (1) the F_2 mapping populations generated from different parent lines, (2) different phenotypic traits studied, and (3) relatively crude estimates of QTL locations and magnitudes in Grillo*et al* . (2009) due to the low resolution arising from smaller F_2 mapping populations and lower density of SSR markers. In addition to a large number loci underlying interspecific divergence of traits, we detected significant epistatic interactions for 23 pairs of QTLs involving 11 traits (Table 3), suggesting that the trait differences between species were highly polygenic and associated with epistasis, pleiotropy and linkage of multiple genes. Interestingly, several important traits that proved to involve reproductive isolation and fitness, including anther length (ANL), first heading (FH), panicle length (PL), and panicle shape (PS) (Cai *et al* ., 2019), were controlled by at least one major-effect QTL (Table 2). This implies that these traits might experience relatively large steps during initial stage of speciation because large-effect loci should be favored when a population is far from the optimum (Orr, 1998a; Connallon & Hodgins, 2021). It is possible, as theory predicted (Orr, 1998a), that the formation of *O. nivara* proceeded in a way of "adaptive walk", in which several large-effect mutations involving adaptive phenotypes took place initially, followed progressively by many small-effect mutations as the phenotypes moved closer to the optimum.

It is noted that the distribution of effect sizes of the QTLs identified in this study followed roughly an exponential model proposed by Orr's (1998a) and that nine out of 12 major-effect loci and as many as 74 small-effect loci underlay 12 putatively adaptive traits (Figs. 4 and 5, Table 2). Such a polygenic and complex genetic basis of adaptive traits and an effect size distribution with significantly skewed toward the right appears to prevail during ecological speciation in plants and animals (e.g., Lexer *et al* ., 2005; Fishman *et al* ., 2002; Nakazato *et al* ., 2013; Lowry *et al* ., 2015; Milano *et al* ., 2016; Ferris *et al* ., 2017; Jacobs *et al* ., 2017; Feng*et al* ., 2019; and reviewed in Dittmar *et al* ., 2016; Hall*et al* ., 2016; Bomblies & Peichel, 2022). Overall, our findings supported accumulating empirical studies that indicated the polygenic basis of adaptive traits and multiple genetic regions underlying trait differentiations during adaptation and speciation (Slate, 2005; Saltz*et al* ., 2017; Nosil *et al* ., 2021; Bomblies & Peichel, 2022).

QTL hotspots and the genetic basis underlying trait divergence

It is hypothesized that mechanisms facilitating coinheritance of adaptive phenotypes are favored when organisms under divergent selection are adapting to multiple different aspects of new environments (Nosil*et al* ., 2021; Bomblies & Peichel, 2022). Despite many studies on plants and animals, empirical investigations on ecological speciation remain rare with limited knowledge on the relationship between clustering of QTLs/genes and phenotypic divergence between species (Archambeault *et al* ., 2020; Bomblies & Peichel, 2022). In this study, we identified 18 QTL hotspots on 11 chromosomes, with each involving multiple traits (3 to 13) and found that 11 of all 12 major-effect QTLs were in the QTL hotspots (Fig. 4, Table S7). These results suggest that the formation of QTL hotspots or coinheritance of loci/genes, particularly the hotspots

involving QTLs with large effect size may play important roles in adaptation and speciation, as evidenced in many plants (Hall *et al* ., 2006; Onishi *et al* ., 2007; Grillo *et al* ., 2009; Lowry *et al* ., 2015; Feris *et al* ., 2017) and animals (Jacobs *et al* ., 2017; Archambeault*et al* ., 2020). Interestingly, the QTLs controlling three RR traits (ANL, FH, and PE) co-localized in the hotspots of chromosomes 1 and 6 simultaneously (Fig. 4, Table S7), suggesting that a series of traits related reproductive isolation were selected together by either tight linkage of loci or pleiotropy. This notion was supported by correlation analyses in which significant correlations were detected between pairwise combinations of ANL, FH, and PE (Fig. 3). QTLs controlling two panicle traits (PL and PS) were also found in multiple hotspots (8 hotspots each) across different chromosomes (Fig. 4, Table S7). Moreover, these traits correlated with almost all other traits (Fig. 3), demonstrating that QTL clustering in which multiple loci evolved together was the preferential strategy of adaptation and speciation (Peichel & Marques, 2017; Nosil *et al* ., 2021).

Early studies indicated that phenotypic differences, of particular the traits involving reproductive isolation, tended to go hand-in-hand (Orr, 2001). Later theoretical (Hoffmann & Rieseberg, 2008; Peichel & Marques, 2017) and empirical studies (Grillo *et al* ., 2009; Linnen*et al* ., 2013; Lowry *et al* ., 2015; Ferris *et al* ., 2017; Archambeault *et al* ., 2020) suggested that the presence of QTL hotspots would facilitate adaptation to new environments and accelerate the process of speciation. A large number of QTL hotspots, as evidenced in our study, suggest that formation of QTL hotspots plays an important role during the ecological speciation at the progress of new species to approach the fitness optimum, although we do not know yet whether these hotspots contain a single pleiotropic locus or many tightly linked loci or both. Elucidating genetic underpinning of QTL hotspots will be critical steps in understanding the molecular mechanisms and the factors facilitating ecological adaptation and speciation.

Divergent selection and its role in ecological speciation of*O. nivara*

It is widely acknowledged that natural selection is the primary force shaping the phenotypic differences that evolve during adaptation and speciation (Orr, 1998b; Rieseberg *et al* ., 2002; Nosil, 2012; Seehausen *et al* ., 2014; Nosil *et al* ., 2021; Schluter & Rieseberg, 2022). Based on previous studies of interspecific phenotypic divergence using variance andQ_{ST}-F_{ST} analyses, Guo*et al* . (2016) found that 9 of 24 phenotypic traits measured showed significant divergence between *O. rufipogon* and *O. nivara* . Similarly, Cai *et al.* (2019) showed that 11 of 18 phenotypic traits exhibited significantly higher values forQ_{ST} than for F_{ST} across six species pairs. These results demonstrated the roles of natural selection rather than random genetic drift in the phenotypic differentiation between *O. rufipogon* and *O. nivara* . However, Q_{ST}-F_{ST} analysis performed under several assumptions (Fraser, 2020) and were unable to distinguish between direct selection acting on the traits and indirect selection due to correlations with other selected traits given that neutral traits correlating with traits under selection may show correlated responses in evolutionary change (Via & Hawthorne, 2005; Muir *et al* ., 2014).

Analysis of the direction of allelic effects of QTLs can facilitate inference on whether trait divergence is consistent with directional natural selection (Orr, 1998b; Rieseberg *et al* ., 2002; Muir*et al* ., 2014). Specifically, if the effects of most QTLs for a trait move the phenotype in the same direction (positive), it is most likely that directional selection has caused the trait divergence. By contrast, weak selection or drift may be the main drivers for the divergence if antagonistic effects are present for the QTLs (negative) (Orr, 1998b; Nakazato *et al* ., 2013; Muir *et al* ., 2014; Ferris *et al* ., 2017). In the present study, we found a high proportion of positive QTLs (Fig. 2), i.e., the direction QTL effects was predominantly in the direction of expected species divergence, suggesting that interspecific differentiations of most traits were driven by divergent natural selection. Moreover, Fraser's v -test statistics were significant for eight traits, supporting the importance of natural selection in phenotypic divergence between *O. rufipogon* and *O. nivara* .

Accumulating evidence indicates that adaptation to new environments often involves shifts of many ecologically important traits and results in covariation of these traits across species (Erickson *et al* ., 2004; Muir *et al* ., 2014; Lowry *et al* ., 2015). In our case, a suite of traits associated the derived *O. nivara* , such as earlier flowering, shorter culm, annual life history and prominently selfing mating system, are most likely to evolve to adapt to drier habitats (Grillo *et al* ., 2009; Banaticla-Hilario *et al* ., 2013; Guo *et al* ., 2016; Cai

et al ., 2019). Trait shifts associated with xeric/mesic divergence have been evident in adaptive adaptation/speciation of many other plant species (e.g., Hall *et al* ., 2006; Lowry *et al* ., 2015; Milano *et al* ., 2016; Ferris *et al* ., 2017). Such trait covariation could be due to coselection, whereby each trait improves the adaptive capacity in dry environments and alternatively, arose from a shared genetic basis (pleiotropy/linkage of genes), in which traits are constrained to evolve in concert (Erickson *et al* ., 2004; Muir *et al* ., 2014). Our previous studies suggested that the change of first heading (FH) might be a primary step during the origin of *O. nivara* , because flowering time contributes to both local adaptation to avoid drought and reproductive isolation to block the gene exchange between species (Guo*et al* ., 2016; Cai *et al* ., 2019; Xu *et al* ., 2020). Interestingly, we found FH in the two largest QTL hotspots with the number of QTLs over 10 (HS1 and HS10) (Fig. 4, Table S7), implying the potential interactions between loci/genes controlling flowering time and those responsible for species divergence of other adaptive traits. Our correlation analyses also observed significantly positive correlation between FH and other adaptive traits (Fig. 3). These findings suggested a common genetic basis underlying the co-adaptation of flowering time and other adaptive traits. Indeed, several studies have cloned/identified the QTLs that pleiotropically control flowering time, plant height, number of spikelets and drought escape in rice (e.g., Yan*et al* ., 2011) and other grass species (Lowry *et al* ., 2015). These co-adaptive traits are therefore excellent candidates for future research in terms of genetic and functional perspectives. Collectively, our QTL analysis added to a growing body of evidence that the annual *O. nivara* evolved from the perennial *O. rufipogon* as an adaptation to new environments due to divergent natural selection that favors co-adapted traits (Grillo *et al* ., 2009; Huang *et al* ., 2013; Guo *et al* ., 2016; Cai *et al* ., 2019). These results are consistent with the argument that adaptation and diversification would proceed rapidly under coordinated selection on multiple traits (Erickson *et al* ., 2004; Saltz *et al* ., 2017). To further investigate the prevalence and molecular basis of genomic coupling may be a key to understanding ecological adaptation and speciation.

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Data availability and Benefit-Sharing

Data and analysis codes are openly available on GitHub at https://github.com/qiang-cg/QTL-wild-rice.Raw sequence data from this study can be found on NCBI Sequence Read Archive (accession no.PRJNA988162).

Author contributions

S. G. conceive and designed project. Q-L. Meng, C-G. Qiang, M-F. Geng, N-N. Ren, M-X. Wang and Z-H. Jiao conducted the field work and phenotyping; Q-L. Meng, C-G. Qiang, J-L Li and X-J. Song performed the QTL mapping; Q-L. Meng, C-G. Qiang, Z. Cai, F-M. Zhang performed data analyses. S. Ge, Q-L. Meng, C-G. Qiang and J-L, Li wrote the manuscript.

Tables and Figures

Table 1. Variation of 19 phenotypic traits measured for *O. rufipogon* and *O. nivara* parental lines in the common garden .

Trait	O. rufipogon	O. rufipogon	O. nivara	O. nivara	t**-statistic**	V**-stati**
	N	mean (SD)	N	mean (SD)		
Reproduction-related trait (RR trait)						
Anther length (ANL)	12	**4.23 (0.22)**	21	1.99 (0.05)	34.912^{***}	12.11^{***}
First heading (FH)	23	**109.48 (6.72)**	21	71 (4.89)	21.847^{***}	8.159^{**}
Panicle exsertion (PE)	12	**8.62 (2.41)**	22	0 (0)	21.464^{***}	5.756^{*}
Habitat-related trait (HR trait)						
Awn length (AWL)	12	2.26 (0.67)	22	**6.38 (0.68)**	-17.057^{***}	2.862
Culm diameter (CD)	12	0.55 (0.07)	21	0.56 (0.07)	-0.465^{ns}	NA
Culm habit (CH)	12	**60.83 (5.97)**	21	36.91 (5.59)	11.34^{***}	6.855^{**}
Culm length (CL)	12	**98.83 (10.35)**	20	57.25 (9.74)	13.615^{***}	6.962^{**}
Flag leaf attitude (FLA)	12	**92.67 (10.67)**	21	36.19 (10.45)	14.736^{***}	17.468^{*}
Flag leaf length (FLL)	12	**28.42 (3.68)**	22	24.18 (2.17)	3.654^{**}	NA
Flag leaf width (FLW)	12	0.8 (0.07)	22	**1.23 (0.08)**	-15.518^{***}	NA
Grain length (GL)	10	8.16 (0.23)	10	**9.17 (0.15)**	-11.603^{***}	2.897
Grain weight (GWE)	10	0.461 (0.02)	10	**0.735 (0.034)**	-21.745^{***}	1.692
Grain width (GWI)	10	2.32 (0.09)	10	**2.82 (0.09)**	-12.759^{***}	6.18^{*}
Panicle length (PL)	12	**26.16 (1.55)**	22	17.98 (1.28)	5.027^{***}	3.053
Panicle shape (PS)	12	**44.25 (5.86)**	21	0 (0)	26.14^{***}	4.965^{*}
Spikelet number (SN)	12	49.08 (7.87)	21	49.29 (4.85)	-0.081^{ns}	NA
Color trait					χ^2	NA
Awn color (AWC)	23	0.52 (0.51)	21	0.43 (0.51)	0.1^{ns}	NA
Basal leaf sheath color (BLSC)	12	1	22	0	29.8^{***}	NA
Stigma color (SC)	12	1	21	0	28.8^{***}	NA

All the quantitative traits except for four in italic (CD, FLL, FLW, and SN) exhibited the differentiation patterns that were same to those found in previous studies of natural populations (Cai *et al* ., 2019; Ren, 2019) and were regarded as to be adaptive. For CD and SN, no significant differentiation was found between parental lines, while for two flag leaf traits (FLL and FLW), the opposite patterns of divergence to those reported for natural populations were determined. Figures in bold face represent larger average values in comparison of the *O. rufipogon* and *O. nivara* parental lines. N , sample size;t -statistic was used for differentiation test between parental lines and v -test was for selection test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. NA, not applicable.

Table 2. Information on the significant QTLs for 16 quantitative traits identified in the F_2

population .

Trait (abbreviation)	**QTL**	**Chr**	**Peak position (cM)**	**LOD**	**Mean RR phenotype**	**Mean RN phenotype**	**Mean NN phenotype**	**Effect and direction**	**PV**
Reproduction-related trait (RR trait)									
Anther length (ANL)	ANL1.a	1	27.61	24.64	0.32	0.302	0.285	0.035	7.6
	ANL1.b	1	144.26	10.23	0.292	0.303	0.307	-0.015	3.0
	ANL2	2	115.71	9.33	0.309	0.303	0.292	0.017	2.7
	ANL3.a	3	20.15	10.93	0.317	0.299	0.291	0.026	3.2
	ANL3.b	3	107.38	5.57	0.299	0.307	0.292	0.007	1.6
	ANL3.c	3	129.58	12.04	0.31	0.302	0.29	0.02	3.5
	ANL4	4	85.27	21.44	0.281	0.302	0.318	-0.037	6.6
	ANL5.a	5	39.03	13.36	0.313	0.302	0.282	0.031	3.9
	ANL5.b	**5**	**114.93**	**45.34**	**0.323**	**0.303**	**0.277**	**0.046**	**15**
	ANL6	6	17.33	18.87	0.312	0.302	0.281	0.031	5.7
	ANL9	9	58.19	16.17	0.318	0.302	0.286	0.032	4.8
First heading (FH)	FH1	1	27.27	7.9	79.323	75.226	72.799	6.524	3.3
	FH3	**3**	**3.51**	**47.03**	**82.376**	**74.014**	**71.811**	**10.565**	**23**
	FH6	**6**	**13.81**	**36**	**79.701**	**74.325**	**72.452**	**7.249**	**17**
	FH7	7	51.53	18.03	71.968	75.552	79.196	-7.228	8.0
	FH12	12	79.93	5.4	77.725	74.007	75.911	1.814	2.2
Panicle exsertion (PE)	PE1	1	4.1	5.63	3.147	3.612	4.471	-1.324	3.0
	PE2	2	120.14	4.42	3.983	3.917	3.151	0.832	2.3
	PE3	3	9.03	4.87	4.406	3.6	3.263	1.143	2.6
	PE4	4	69.21	5.42	3.22	3.528	4.574	-1.354	2.9
	PE6.a	6	17.24	7.67	4.778	3.586	2.389	2.389	4.1
	PE6.b	6	67.69	9.37	4.568	3.78	2.336	2.232	5.1
	PE9	9	42.55	11.14	5.229	3.546	2.844	2.385	6.1
	PE12	12	91.56	5.64	4.312	3.702	2.927	1.385	3.0
Habitat-related trait (HR trait)									0
Awn length (AWL)	AWL1.a	1	14.14	21.86	4.1	4.649	5.129	1.029	6.3
	AWL1.b	1	145.27	10.31	4.413	4.67	4.841	0.428	2.8
	AWL2	2	63.13	11.61	4.3	4.636	5.019	0.719	3.2

Trait (abbreviation)	**QTL**	**Chr**	**Peak position (cM)**	**LOD**	**Mean RR phenotype**	**Mean RN phenotype**	**Mean NN phenotype**	**Effect and direction**	**PV**
	AWL3.a	3	47.99	21.95	4.195	4.648	5.19	0.995	6.3
	AWL3.b	3	110.83	11.93	4.184	4.783	4.941	0.757	3.3
	AWL4	**4**	**83.6**	**89.48**	**3.177**	**4.846**	**5.528**	**2.351**	**34**
	AWL6.a	6	15.32	20.72	5.203	4.593	3.949	-1.254	5.9
	AWL6.b	6	125.08	8.81	4.388	4.623	5.003	0.615	2.4
	AWL7	7	109.29	10.47	4.507	4.648	4.843	0.336	2.8
	AWL12	12	9.62	6.49	4.758	4.702	4.454	-0.304	1.7
Culm diameter (CD)	CD1	1	26.44	7.46	0.473	0.467	0.494	0.021	4.6
	CD3	3	101.78	10.55	0.496	0.474	0.455	-0.041	6.6
	CD6	6	101.14	10.12	0.457	0.475	0.497	0.04	6.3
	CD8	8	84.44	7.73	0.493	0.469	0.464	-0.029	4.7
	CD9	9	64.89	5.18	0.458	0.473	0.493	0.035	3.1
Culm habit (CH)	CH2	2	108.69	6.8	42.153	45.795	47.981	-5.828	4.2
	CH4	4	106.77	5.33	47.264	46.473	41.429	5.835	3.3
	CH5	5	96.86	7.45	48.359	46.09	42.134	6.225	4.7
	CH6	6	13.14	5.16	45.848	46.506	41.99	3.858	3.2
	CH8	8	70.4	4.38	47.995	44.184	45.202	2.793	2.7
Culm length (CL)	CL1	1	50.37	7.36	69.522	74.887	76.363	-6.841	3.9
	CL2	2	129.93	8.46	77.358	74.814	70.236	7.122	4.5
	CL5	5	117.86	5.62	75.687	74.646	70.426	5.261	2.9
	CL6.a	6	14.82	15.78	79.327	73.651	65.28	14.047	8.6
	CL6.b	6	67.69	8.68	76.803	74.508	67.797	9.006	4.6
	CL9	9	79.36	7.5	72.574	73.023	76.157	-3.583	3.9
	CL10	10	28.09	5.9	77.651	73.224	71.832	5.819	3.1
	CL11	11	69.08	4.54	70.277	75.432	74	-3.723	2.3
Flag leaf attitude (FLA)	FLA1	1	132.81	5.89	61.25	58.659	54.076	7.174	3.8
	FLA3	3	4.43	6.18	63.372	56.823	55.198	8.174	4.0
	FLA4	4	87.86	5.59	62.864	57.433	54.586	8.278	3.6
	FLA8	8	26.31	4.53	59.769	59.094	53.185	6.584	2.9
	FLA11	11	63.97	4.3	54.593	58.069	60.862	-6.269	2.7
Flag leaf length (FLL)	FLL1	1	13.13	5.83	20.081	20.831	21.741	-1.66	3.0
	FLL6	**6**	**16.15**	**26.3**	**22.559**	**20.761**	**18.548**	**4.011**	**14**
	FLL8	8	86.2	6.07	21.666	20.846	19.967	1.699	3.1
	FLL9	9	54.52	17.07	19.459	20.763	22.254	-2.795	9.2
	FLL10	10	83.8	4.99	19.915	20.91	21.822	-1.907	2.5

Trait (abbreviation)	**QTL**	**Chr**	**Peak position (cM)**	**LOD**	**Mean RR phenotype**	**Mean RN phenotype**	**Mean NN phenotype**	**Effect and direction**	**PV**
	FLL12	12	100.26	7.22	21.923	20.459	20.624	1.299	3.7
Flag leaf width (FLW)	**FLW1**	**1**	**23**	**46.75**	**0.836**	**0.892**	**1.007**	**-0.171**	**25**
	FLW2	2	20.83	7.41	0.891	0.898	0.949	-0.058	3.4
	FLW3	3	51.76	8.46	0.94	0.91	0.869	0.071	3.9
	FLW5	5	95.19	8.16	0.893	0.905	0.93	-0.037	3.7
	FLW7	7	111.13	5.32	0.879	0.915	0.934	-0.055	2.4
	FLW9	9	64.22	10.18	0.879	0.895	0.958	-0.079	4.7
Grain length (GL)	GL1.a	1	26.19	11.33	8.995	8.863	8.7	-0.295	5.1
	GL1.b	**1**	**160.28**	**20.38**	**8.661**	**8.839**	**9.037**	**0.376**	**9.6**
	GL2.a	2	27.13	12.99	8.971	8.827	8.765	-0.206	5.9
	GL2.b	2	110.7	10.03	8.912	8.868	8.759	-0.153	4.5
	GL3.a	3	44.48	6.51	8.88	8.878	8.751	-0.129	2.9
	GL3.b	3	121.97	17.79	8.715	8.837	9.016	0.301	8.3
	GL6	6	98.3	11.88	8.738	8.875	8.917	0.179	5.4
	GL12	12	81.02	11.93	8.763	8.909	8.818	0.055	5.4
Grain weight (GWE)	GWE1.a	1	5.1	8.07	0.581	0.599	0.608	0.027	5.1
	GWE1.b	1	159.95	7.04	0.578	0.597	0.613	0.035	4.4
	GWE2	2	48.5	9.41	0.613	0.597	0.582	-0.031	6.0
	GWE10	10	69.92	10.48	0.584	0.6	0.602	0.018	6.7
Grain width (GWI)	**GWI1**	**1**	**23.42**	**26.44**	**2.369**	**2.429**	**2.534**	**0.165**	**15**
	GWI4.a	4	12.47	13.69	2.496	2.439	2.381	-0.115	7.8
	GWI4.b	4	99.66	5.51	2.405	2.45	2.467	0.062	3.0
	GWI8	8	47.66	7.08	2.441	2.43	2.481	0.04	3.9
Panicle length (PL)	**PL1**	**1**	**22.84**	**36.96**	**18.716**	**19.505**	**21.283**	**-2.567**	**14**
	PL2	2	107.52	7.9	20.456	19.791	19.18	1.276	2.7
	PL3	3	2.09	16.06	20.566	19.558	19.495	1.071	5.6
	PL4	4	83.43	12.8	18.503	20.057	20.325	-1.822	4.4
	PL5	5	92.18	11.25	20.829	19.845	18.814	2.015	3.9
	PL6	**6**	**15.74**	**37.92**	**21.294**	**19.645**	**17.747**	**3.547**	**14**
	PL7	7	7.61	5.62	19.741	19.572	20.146	-0.405	1.9
	PL9	9	65.31	14.55	19.207	19.576	20.583	-1.376	5.1
	PL10	10	20.65	12.04	20.498	19.834	19.094	1.404	4.2
	PL11	11	84.09	7.55	19.453	19.559	20.655	-1.202	2.5
Panicle shape (PS)	PS1	1	23	11.16	11.734	13.669	20.933	-9.199	5.1
	PS3	3	44.56	7.96	19.238	14.548	11.868	7.37	3.6

Trait (abbreviation)	QTL	Chr	Peak position (cM)	LOD	Mean RR phenotype	Mean RN phenotype	Mean NN phenotype	Effect and direction	PV
	PS5	5	112.92	8.99	19.768	14.526	12.038	7.73	4.1
	PS6.a	**6**	**15.15**	**24.4**	**21.16**	**15.367**	**4.121**	**17.039**	**11**
	PS6.b	6	71.54	6.4	18.15	15.358	10.551	7.599	2.9
	PS6.c	6	106.83	7.79	10.92	17.016	15.153	-4.233	3.5
	PS7	7	111.8	7.41	9.918	16.734	17.91	-7.992	3.3
	PS8	8	64.63	17.04	19.186	15.324	9.062	10.124	8.0
Spikelet number (SN)	**SN1.a**	**1**	**22.84**	**64.74**	**34.619**	**38.09**	**49.867**	**-15.248**	**25**
	SN1.b	1	139.17	10.23	44.343	40.037	37.228	7.115	3.2
	SN3	3	62.72	21.75	44.791	39.529	36.534	8.257	7.3
	SN5	5	36.19	5.43	41.68	40.724	37.53	4.15	1.7
	SN6.a	6	20.09	15.47	43.65	39.558	37.327	6.323	5.0
	SN6.b	6	105.24	8.04	37.875	40.341	43.102	-5.227	2.5
	SN7.a	7	19.57	12.1	38.791	39.343	42.563	-3.772	3.9
	SN7.b	7	66.26	8.82	40.834	41.089	37.595	3.239	2.8
	SN9	9	59.95	26.15	35.908	39.488	45.385	-9.477	8.9
	SN12	12	93.73	5.12	42.158	39.328	40.246	1.912	1.6

All traits except for four in italic were regarded as to be adaptive because they showed significant and same differentiation patterns to those reported in previous studies of large samples/populations. PVE (%), percent phenotypic variance explained by QTL. The QTLs with major effect size (PVE > 10%) are in bold. Effect size was estimated as the difference between homozygous *O. rufipogon*alleles and homozygous *O. nivara* alleles at the QTL, with a positive value if the effect was consistent with species divergence and a negative value if the effect was opposite of species divergence.

Table 3. Significant epistatic interactions between QTLs for 16 quantitative traits revealed in the F_2population .

Trait	QTLs	LOD	PV
Reproduction-related trait (RR trait)	Reproduction-related trait (RR trait)	Reproduction-related trait (RR trait)	
ANL	ANL1.a×ANL9	2.72	0.7
	ANL3.c×**ANL5.b**	3.58	1.0
FH	**FH3**×**FH6**	18.21	8.0
	FH3×FH7	2.66	1.1
	FH6×FH7	3	1.2
Habitat-related trait (HR trait)	Habitat-related trait (HR trait)		
AWL	AWL1.a×AWL2	2.81	0.7
	AWL1.b×AWL3.a	3.61	0.9
	AWL3.a×AWL6.a	2.46	0.6
	AWL4×AWL6.b	3.2	0.8
	AWL6.a×AWL7	2.96	0.7
CL	CL6.b×CL9	2.53	1.3
CD	CD1×CD6	2.3	1.3
	CD3×CD8	2.87	1.7
FLW	**FLW1**×FLW5	3.3	1.4
GL	GL2.a×GL2.b	3.98	1.7

Trait	QTLs	LOD	PV
	GL3.b×GL12	2.29	1.0
	GL6×GL12	2.57	1.1
GWE	GWE1.a×GWE10	3.4	2.1
	GWE2×GWE10	3.2	2.0
GWI	GWI4.a×GWI8	2.36	1.2
PL	**PL1**×PL3	2.5	0.8
	PL1×PL4	2.26	0.7
PS	PS1×PS8	3.8	1.7

Those with bold face indicate the QTLs with major effect. *, $P< 0.05$; **, $P < 0.01$; ***, $P< 0.001$.

Figures

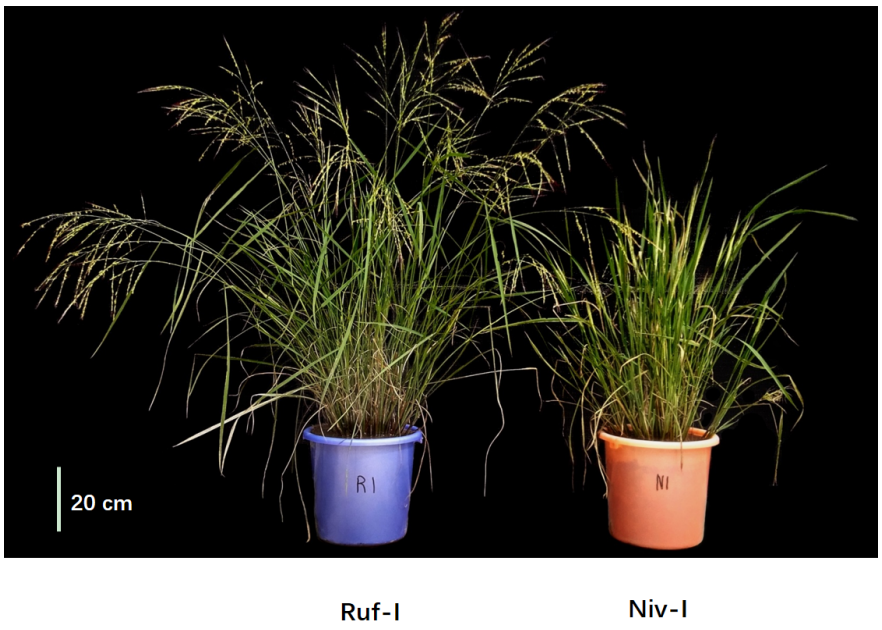

Figure 1. Gross morphology of the *O. rufipogon*(Ruf-I) and *O. nivara* (Niv-I) parental lines used to generate F_2 mapping population.

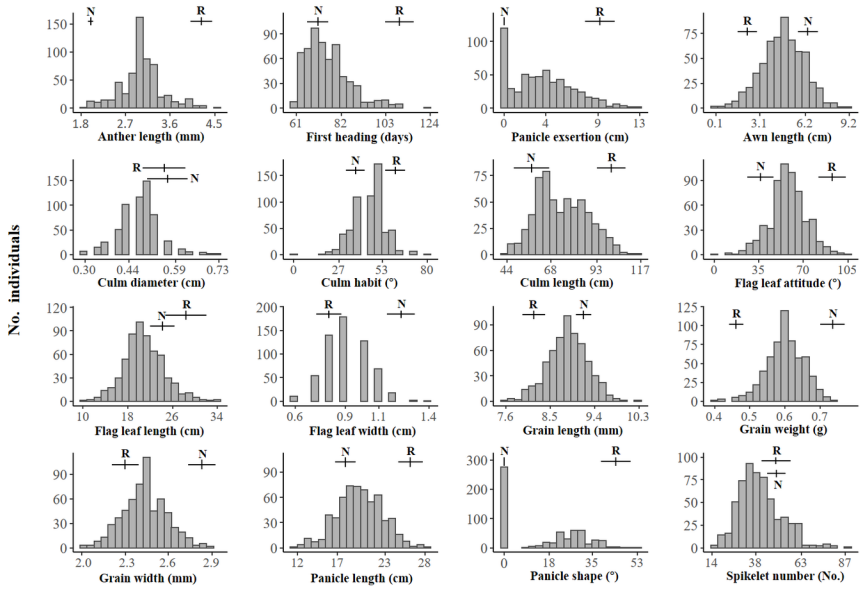

Figure 2. Frequency distribution of 16 quantitative traits in the *O. rufipogon* (R) and *O. nivara* (N) parental lines and F_2 population. Means and standard deviations of two parental lines are indicated by vertical and horizontal lines, respectively.

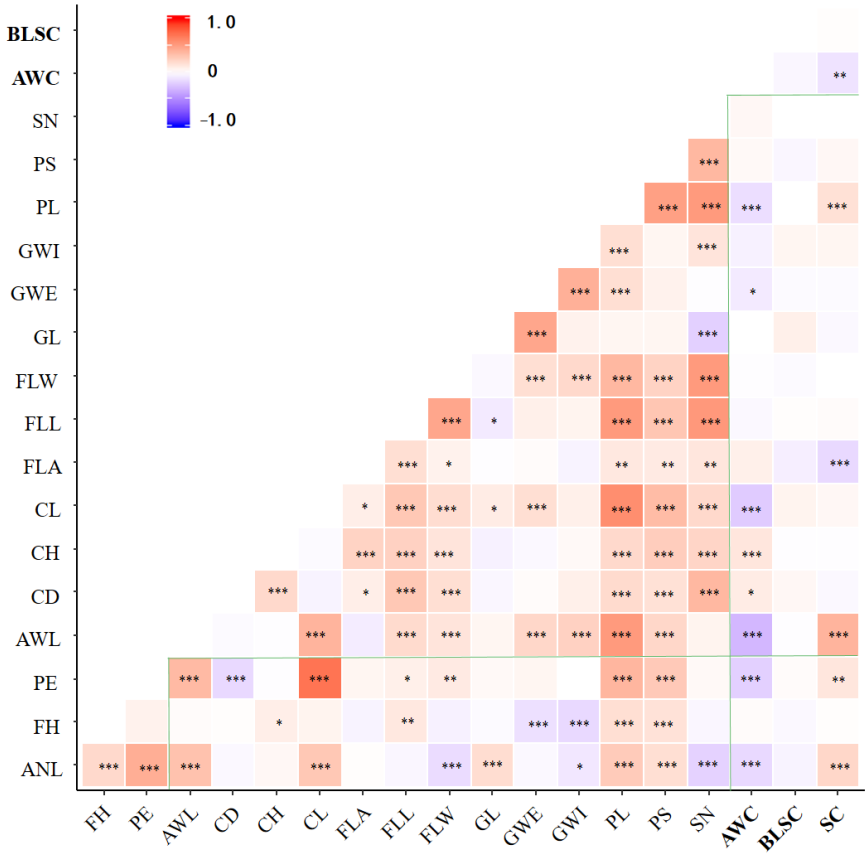

Figure 3. Pairwise correlation of 19 traits in the *O. rufipogon* × *O. nivara* F_2 populations. Three color (qualitative) traits were in bold. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. The full names for the trait abbreviations are the same to those in Table 1.

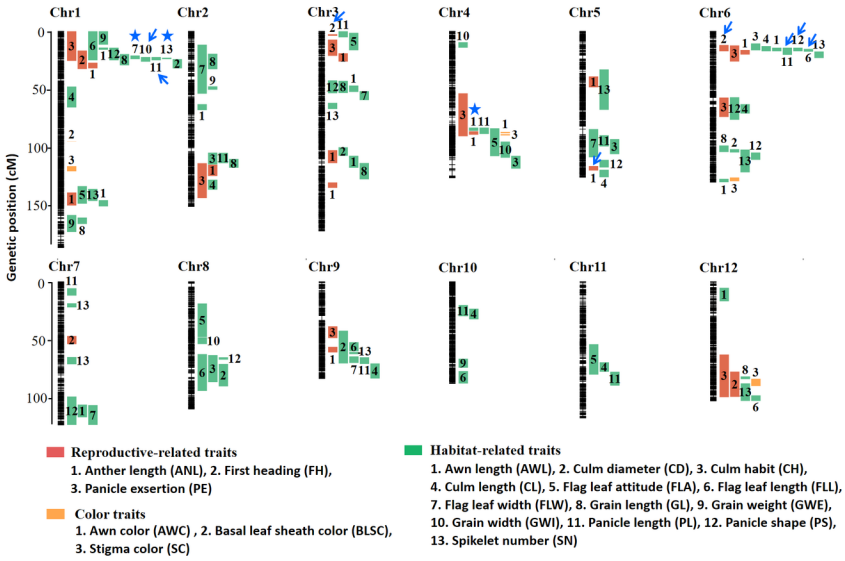

Figure 4. Genetic map of the *O. nivara* × *O. rufipogon* F_2 population, with the QTL locations involving all 19 phenotypic traits presented. Rectangular box indicates 1.5-LOD confidence intervals of each QTL, with width of the boxes corresponding to the range of genomic regions. Number within/beside the boxes represents the QTLs illustrated in the legends: reproduction-related traits (RR traits) (red), habitat-related traits (HR traits) (green) and color traits (yellow). Arrows and stars above the numbers stand for the major-effect and large-effect QTLs, respectively. QTLs on the map are ordered according their positions on chromosome.

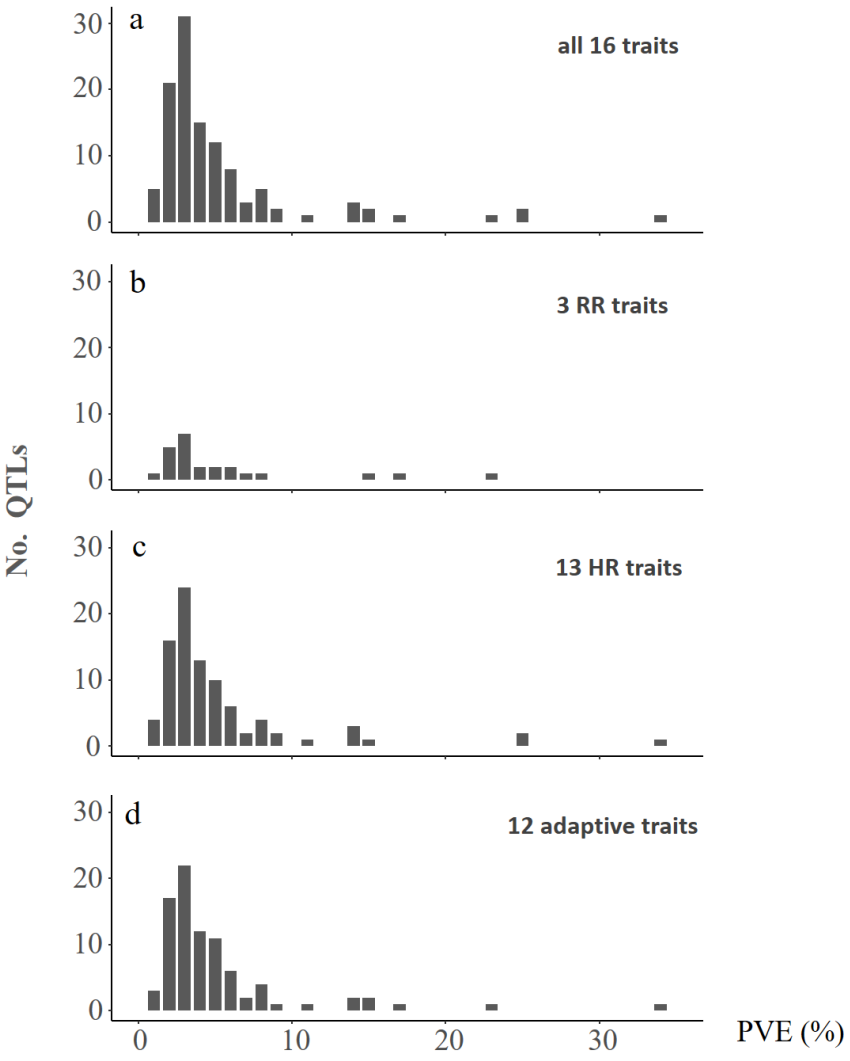

Figure 5. The distribution of effect sizes of QTLs identified in the F_2 mapping populations. (a) all 16 quantitative traits; (b) three RR traits; (c) 13 HR-traits; and (d) 12 putatively adaptive traits.