Genome features of common vetch (*Vicia sativa*) in natural habitats

Running title: **The genome of common vetch**

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Highlight

Sequence analysis of the common vetch (*Vicia sativa*) genome and SNP genotyping across natural populations revealed nucleotide diversity levels associated with native population environments.

Abstract

Wild plants are often tolerant to biotic and abiotic stresses in their natural environments, whereas domesticated plants such as crops frequently lack such resilience. This difference is thought to be due to the high levels of genome heterozygosity in wild plant populations and the low levels of heterozygosity in domesticated crop species. In this study, common vetch (*Vicia sativa*) was used as a model to examine this hypothesis. The common vetch genome (2n = 14) was estimated as 1.8 Gb in size. Genome sequencing produced a reference assembly that spanned 1.5 Gb, from which 31,146 genes were predicted. Using this sequence as a reference, 24,118 single nucleotide polymorphisms were discovered in 1,243 plants from 12 natural common vetch populations in Japan. Common vetch genomes exhibited high heterozygosity at the population level, with lower levels of heterozygosity observed at specific genome regions. Such patterns of heterozygosity are thought to be essential for adaptation to different environments. These findings suggest that high heterozygosity at the population level would be required for wild plants to survive under natural conditions while allowing important gene loci to be fixed to adapt the conditions. The resources generated in this study will provide insights into *de novo* domestication of wild plants and agricultural enhancement.

Keywords: Common vetch; ddRAD-Seq; Genome sequence; Natural populations; Nucleotide diversity; Single nucleotide polymorphism

Introduction

Wild plants, including weeds that have not yet been domesticated or cultivated, generally possess characteristics that allow them to survive and propagate in their natural environments when challenged by local biotic and abiotic stresses (Mammadov *et al.*, 2018). The resilience exhibited by wild plants is thought to be due to their high levels of genetic heterogeneity (Canc□ado, 2011). Indeed, genetic heterogeneity was effective in suppressing disease when populations of genetically diversified crops were planted together in the same fields (Zhu *et al.*, 2000).

In contrast with wild plants, crop plants have lost their natural survival traits as a result of the extremely low levels of genetic heterogeneity found in monoculture crop species (Mundt, 2002). Therefore, disease-, insect-, and weed-controls are essential in commercial crop cultivation to reduce losses and maximize yields. This requires additional crop management costs for farmers, for example, for labor and agrochemicals. There are two main reasons for the low genetic heterogeneity in crop species. One reason is crop domestication (Izawa *et al.*, 2009), in which only a few plants possessing desirable phenotypes, such as large fruit size, non-seed shattering, and long-seed dormancy, are selected from the broad genetic pools of wild plants. The second reason is selective breeding for desirable traits. While valuable for stabilizing crop phenotypes such as yield, these selective processes have reduced genetic diversity in monoculture crops by purging diverse germplasms (Fu, 2015). During domestication and selective breeding, small numbers of alleles that have large effects on phenotypic variations have often been targeted, further reducing the genetic diversity within cultivated varieties (Fernie and Yan, 2019).

While remaining more diverse than crop species, wild plant populations have also experienced loss of genetic heterogeneity at some loci, though in wild plants this is due to directional selection and genetic drift. For example, natural populations of Arabidopsis have lost genetic heterogeneity at flowering loci to synchronize flowering time (Mendez-Vigo *et al.*, 2011), which is beneficial for propagation under natural conditions. This suggests that genome-wide genetic heterogeneity is not necessarily required for wild plant populations and that small numbers of loci could become fixed under certain selective conditions. This suggests that it would be possible to generate new plant populations with a) fixed domestication loci with suitable alleles for agricultural traits and b) high general levels of genetic diversity elsewhere in the genome. Such plant populations could be used as crop species, as proposed by Litrico and Violle (Litrico and Violle, 2015), and would possess natural resistance and suppression traits, as a result of high heterogeneity, that would enhance population resilience to biotic and abiotic stresses. As favorable agricultural alleles would be fixed, the benefits of genetic heterogeneity would exist alongside desirable agricultural traits. Mixtures of heterozygous plant populations have already been used as crops in allogamous species such as onion and clover. However, the potential benefits of genetic heterogeneity for autogamous plants such as legumes remain unclear.

Common vetch (*Vicia sativa*), a wild legume commonly found in open fields, was partially domesticated and cultivated in the past (Bryant and Hughes, 2011). Common vetch therefore has crop potential and can serve as a model for examination of genetic heterogeneity and domestication. The first step is to evaluate the levels of genetic heterogeneity in wild common vetch populations. However, no genome sequence data is available in common vetch. At least three different chromosome numbers (2n = 10, 12, and 14) have been reported (Ladizinsky, 1998; Ladizinsky and Waines, 1982). In this study, a reference sequence for common vetch was developed and single nucleotide polymorphism (SNP) analysis with double-digest restriction-site associated DNA sequencing (ddRAD-Seq) was used to evaluate heterogeneity in genomes of common vetch populations.

Materials and methods

Plant materials

A standard inbred line of common vetch (*V. sativa*), KSR5, was established from a wild plant collected from Kisarazu, Chiba, Japan, by self-pollination for more than three generations. KSR5 was used for genome and transcriptome sequencing analysis. For genetic diversity analysis, 1,243 plants were collected from 12 locations across the latitude from 31.3ºN to 38.8ºN in Japan (Figure 1, Supplementary Table S1). In addition, eight accessions from France, Germany, Greece, Iran, Italy, and Tunisia were obtained from the NIAS Genebank, Tsukuba, Japan (Supplementary Table S1). Genomic DNA was extracted from young leaves with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Chromosome observation

Root tips of two-day-old seedlings of KSR5 were treated with 0.05% colchicine for 18 hours, fixed with 1:3 acetate:ethanol for 2 hours, and washed three times with water. Cell walls of the root tips were digested with 2% cellulase (SERVA Electrophoresis GmbH, Heidelberg, Germany), 2% macerozyme (SERVA Electrophoresis GmbH), and 0.1 M sodium acetate for four hours at 37ºC. The root tip cells spread on a slide glass were fixed again with 1:3 acetate:ethanol and dried at room temperature. Chromosomes were stained with 1 ug/mL DAPI (4,6-Diamidino-2-phenylindole) in Fluoro-KEEPER Antifade Reagent (Nacalai Tesque, Kyoto, Japan) and were observed under a confocal laser scanning microscope, LSM700 (Carl Zeiss, Oberkochen, Germany). Chromosome length was measured with ImageJ (Schneider *et al.*, 2012).

Sequencing analysis of the common vetch genome

Genomic DNA from KSR5 was used to construct one paired-end (insert size of 500 bp) and four mate-pair sequencing libraries (insert sizes of 2, 5, 10, and 15 kb) in accordance with manufacturer protocols (Illumina, San Diego, CA, USA). Libraries were then sequenced using a HiSeq2000 instrument (Illumina). A long insert library for KSR5 was also prepared and sequenced on an RSII instrument (PacBio, Menlo Park, CA, USA). The paired-end sequence reads were used for genomic size estimation based on *k*-mer frequency (*k* = 17) using Jellyfish (Marcais and Kingsford, 2011). The paired-end and mate-pair reads were assembled and scaffolded with SOAPdenovo2 (Luo *et al.*, 2012). Gaps, represented by Ns in the scaffold sequences, were filled by PBjelly (English *et al.*,

2012) with PacBio reads, in which sequence errors were corrected with the paired-end reads by proovread (Hackl *et al.*, 2014). Contaminated sequences were removed by BLASTN search (Altschul *et al.*, 1990), with an E-value cutoff of 1E-10 and length coverage of ≥10%, against sequences from potential contaminating resources such as organelles (the plastid and mitochondrion genome sequences of *L. japonicus* and *V. faba*: KF042344, AP002983, JN872551, and KC189947), bacteria and fungi (NCBI bacteria and fungi), human (hg19), and artificial sequences (UniVec and PhiX). The resulting sequences that were ≥1,000 bp in size were selected and designated VSA_r1.0 as a draft common vetch genome. Completeness of the assembly was assessed with sets of a Benchmarking Universal Single-Copy Orthologs (BUSCO) (Simao *et al.*, 2015).

RNA sequencing and assembly

Total RNA was extracted from ten tissue samples (roots, seedlings, stems, apical buds, immature and mature leaves, tendrils, flower buds, flowers, and pods) using an RNeasy Mini Kit (Qiagen) and treated with RQ1 RNase-Free DNase (Promega, Madison, WI, USA) to remove contaminating genomic DNA. RNA libraries were constructed in accordance with the TruSeq Stranded mRNA Sample Preparation Guide (Illumina). Nucleotide sequences were obtained with a MiSeq instrument (Illumina) in the paired-end 301 bp mode. Low-quality reads were removed using PRINSEQ (Schmieder and Edwards, 2011) and adapter sequences were trimmed with fastx_clipper (parameter, -a AGATCGGAAGAGC) in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit). The resulting reads were assembled using Trinity (Grabherr *et al.*, 2011) with parameters of –min_contig_length 100, –group_pairs_distance 400, and –SS_lib_type RF to generate a non-redundant gene sequence set.

Repetitive sequence and RNA coding gene analysis

A *de novo* repeat sequence database for VSA_r1.0 was built using RepeatScout (Price *et al.*, 2005) (version 1.0.5). Repetitive sequences in VSA_r1.0 were searched for using RepeatMasker (version

4.0.3) (http://www.repeatmasker.org) based on known repetitive sequences registered in Repbase (Bao *et al.*, 2015) and the *de novo* repeat libraries. Transfer RNA genes were predicted using tRNAscan-SE (version 1.23) (Chan and Lowe, 2019) with the default parameters, and ribosomal RNA (rRNA) genes were predicted using BLASTN searches with an E-value cutoff of 1E-10, with the *Arabidopsis thaliana* 18S rRNA (accession number: X16077) and 5.8S and 25S rRNAs (accession number: X52320) used as query sequences.

Protein-coding gene prediction and annotation

Putative protein-coding genes in VSA_r1.0 were identified with a MAKER pipeline (version 2.31.8) (Cantarel *et al.*, 2008) including *ab-initio*-, evidence-, and homology-based gene prediction methods. For this prediction, the non-redundant gene sequence set generated from the RNA-Seq analysis and peptide sequences predicted in the genomes of four Fabaceae members, namely, *Arachis duranensis* (V14167.a1.M1) (Bertioli *et al.*, 2016), *Lotus japonicus* (rel. 3.0) (Sato *et al.*, 2008), *Medicago truncatula* (4.0v1) (Young *et al.*, 2011), and *Phaseolus vulgaris* (v1.0) (Schmutz *et al.*, 2014), were used as a training data set. In addition, BRAKER1 (version 1.3) (Hoff *et al.*, 2016) was used to complete the gene set for VSA_r1.0. Genes related to transposable elements (TEs) were detected using BLASTP searches against the NCBI non-redundant (nr) protein database with an E-value cutoff of 1E-10 and by using InterProScan (version 4.8) (Jones *et al.*, 2014) searches against the InterPro database with an E-value cutoff of 1.0.

Putative VSA_r1.0 genes were clustered using CD-hit (version 4.6.1) (Li and Godzik, 2006) with the UniGene set of the four Fabaceae members as above with the parameters c□ = □0.6 and aL = 0.4. The predicted genes were annotated with plant gene ontology (GO) slim categories and euKaryotic clusters of Orthologous Groups (KOG) categories (Tatusov *et al.*, 2003), and mapped onto the Kyoto Encyclopedia of Genes and Genomes (KEGG) reference pathways (Ogata *et al.*, 1999).

Gene expression was quantified by mapping the RNA-Seq reads onto VSA_r1.0 using HISAT2 (Kim *et al.*, 2015) followed by normalization to determine fragments per kilobase of exon per million mapped fragments (FPKM) values using StringTie (Pertea *et al.*, 2015) and Ballgown (Frazee *et al.*, 2015) in accordance with the published protocol (Pertea *et al.*, 2016).

Genetic diversity analysis

Genome-wide sequence variations in wild vetch populations were analyzed by a double-digest restriction-site associated DNA sequencing (ddRAD-Seq) technique (Peterson *et al.*, 2012). In accordance with the workflow established in our previous study (Shirasawa *et al.*, 2016), genomic DNA samples from each line were digested with the restriction enzymes *Pst*I and *Eco*RI to prepare ddRAD-Seq libraries, which were then sequenced on a HiSeq2000 (Illumina) instrument in paired-end 93 bp mode. Low-quality sequences were removed and adapters were trimmed using PRINSEQ (Schmieder and Edwards, 2011) and fastx_clipper in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit), respectively. The remaining high-quality reads were mapped onto VSA_r1.0 as a reference using Bowtie2 (Langmead and Salzberg, 2012). The resultant sequence alignment-map format (SAM) files were converted to binary sequence alignment-map format (BAM) files and subjected to SNP calling using the mpileup option of SAMtools (Li *et al.*, 2009) and the view option of BCFtools. High-confidence SNPs were selected using VCFtools (Danecek *et al.*, 2011) with the following criteria: (1) depth of coverage ≥5 for each line, (2) SNP quality scores of 999 for each locus, (3) minor allele frequency ≥0.05 for each locus, and (4) proportion of missing data <0.5 for each locus. The effects of SNPs on gene function were predicted using SnpEff v4.2 (Cingolani *et al.*, 2012).

Nucleotide divergency (π) values and heterozygosity levels for SNP sites of each population were calculated using the site-pi and het options in VCFtools (Danecek *et al.*, 2011), respectively. Principal component analysis (PCA) was performed to determine the relationships among samples using TASSEL (Bradbury *et al.*, 2007) and population structure was investigated using

ADMIXTURE (Alexander *et al.*, 2009). The R package WGCNA (Langfelder and Horvath, 2008) was used for SNP module detection.

Results

Chromosome number of a common vetch line, KSR5

A total of 14 chromosomes, including two mini chromosomes, were observed in metaphase cells of root tips of the standard inbred line, KSR5 (Figure 2, Table 1). Relative length of the chromosomes was measured in five cells and sorted by the length order. In accordance with the chromosome length, the 14 chromosomes were grouped into seven pairs (I to VII), suggesting that the genome of KSR5 was 2n = 14. The relative length of the longest chromosome (I) was 22.3% of the total length of haploid genome, followed by 21.0% (II), 18.6% (III), 16.1% (IV), 10.3% (V), 9.3% (VI), and 2.7% (VII).

Sequencing and genome assembly

The standard inbred line of common vetch (*V. sativa*), KSR5, was sequenced. In total, 1.8 billion paired-end reads corresponding to 186.7 Gb (Supplementary Table S2) were obtained. The distribution of distinct *k*-mers (*k* = 17) showed a single main peak at multiplicities of 78 with minor peaks (Figure 3). The size of the common vetch genome was estimated to be 1,769 Mb. The paired-end reads (105× genome coverage) were assembled with mate-pair reads of four libraries (146× genome coverage in total) to obtain 6,487 thousand (k) scaffold sequences of total length 2.5 Gb with an N50 of 30.5 kb. After removing 6,421 k contaminated sequences and short scaffolds (<1 kb), sequence gaps presented by Ns in the remaining sequences were filled with PacBio long reads (3× genome coverage) to obtain a draft sequence of the common vetch genome, namely, VSA_r1.0. The total length of VSA_r1.0 was 1,541 Mb and consisted of 54,083 sequences with an N50 of 90.1 kb (Table 2). Although 513 k gaps occupied 501 Mb in total (32.5%), the gene space was well represented in accordance with BUSCO examination, indicating 94.1% ortholog completion.

Repeat sequence analysis

Sequences totaling 782 Mb (51.9%) were identified as repeat elements such as transposons and retrotransposons (Table 3). Of this, sequences totaling 267 Mb were repeat sequences reported in other organisms, and sequences in the remaining 531 Mb were uniquely identified in VSA_r1.0. Of the previously reported repeats, long terminal repeat retroelements were predominant (200 Mb). Furthermore, 109,151 simple-sequence repeats with 52,874 di-, 39,198 tri-, 12,354 tetra-, 3,414 penta-, and 1,311 hexa-nucleotide repeat motifs were also found.

Gene prediction and annotation

In total, 31,146 protein-encoding genes, with average length of 1,008 bp and N50 of 1,419 bp, were predicted in VSA_r1.0 (Table 2). For the evidence-based MAKER pipeline, 166 million (M) RNA reads from ten tissue samples (Supplementary Table S2) were assembled into 181,211 transcribed sequences and used to predict 27,880 genes (genes with .mk suffix). A further 3,266 genes were predicted using an *ab-initio*-based method (genes with .br suffix). GO classification assigned 8,878, 4,059, and 13,752 genes to the GO slim terms of biological process, cellular component, and molecular function, respectively (Supplementary Table S3). KOG analysis revealed 2,766, 4,888, and 4,424 genes with significant similarities to genes involved in information storage and processing, cellular processing and signaling, and metabolism, respectively (Supplementary Table S4). Finally, 1,720 genes were mapped to KEGG metabolic pathways (Supplementary Table S5). Gene clustering analysis revealed 5,566 gene clusters that were common to the five legume species tested (*V. sativa*, *A. duranensis*, *L. japonicus*, *M. truncatula*, and *P. vulgaris*) and 12,321 clusters that were unique to common vetch (Figure 4). In addition to mRNA sequences, 58 rRNA- and 1,437 tRNA-encoding genes were predicted.

Single nucleotide polymorphisms in natural populations

Genome-wide SNPs were identified across the 12 common vetch populations from Japan, consisting of 1,243 lines, and eight lines from France, Germany, Greece, Iran, Italy, and Tunisia from the NARO GeneBank (Tsukuba, Japan) (Supplementary Table S1). Approximately 1.1 million ddRAD-Seq reads per sample were obtained (Supplementary Table S2) and 84.4% of the reads aligned to the VSA_r1.0 reference sequence. The ddRAD-Seq reads covered 2.4 Mb (0.16%) of the reference assembly with ≥5 reads. Sequence alignments detected 46,715 high-confidence SNPs (30.9% transitions and 69.1% transversions). SNP density was calculated as 1 SNP per 51 bp. When only the 12 populations from Japan were considered, the number of SNPs decreased to 24,118 (1 SNP per 100 bp), ranging from 4,709 SNPs in the SDI population (1 SNP per 510 bp) to 10,040 SNPs in the ABK population (1 SNP per 239 bp) (Table 4).

PCA and admixture analysis indicated that there were 2–11 subpopulations in each of the 12 populations from Japan (Figure 5, Table 3, Supplementary Figures S1). The observed heterozygosity scores were lower than the expected values (Table 4). Nucleotide divergency scores (π) at SNP sites were similarly distributed across ten of the populations from Japan, with median values of 0.31–0.34. The remaining two populations, NGT and SDI, exhibited median values of ~0.25 (Table 4). Of the 46,715 high-confidence SNPs, 24,118 clustered according to their π scores to generate 82 modules (Supplementary Figure S2). Of these, the π scores of one cluster, ‘cyan’, which contained 190 SNPs, negatively correlated with the latitude of sampling location (Figure 1 and 6). In total, 88 genes were associated with the 190 SNPs, and one of the genes (Vsa_sc30698.1_g030.1.mk) showed sequence similarity to the Arabidopsis gene for a MADS-box protein, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1), known to be involved in the flowering pathway in plants. Vsa_sc30698.1_g030.1.mk was predominantly transcribed in tendrils (FPKM = 5.0) followed by apical buds (0.5) and stems (0.4), whereas no expression was observed in the other seven tissues, i.e., roots, seedlings, immature and mature leaves, flower buds, flowers, and pods.

Discussion

A draft common vetch (*V. sativa*) genome sequence was generated in this study. Although several legume genome sequences were released previously (Bauchet *et al.*, 2019), this is the first report of a genome from the genus *Vicia*, which contains several agronomically important legume crops such as fava bean (*V. faba*). *Vicia* genomes are large (e.g., 1.8 Gb for *V. sativa* and 13 Gb for *V. faba*) due to their massive repetitive sequences, including TEs (Bryant and Hughes, 2011; Hill *et al.*, 2005; Nouzova *et al.*, 2001; Pearce *et al.*, 1996), hampering *de novo* genome assembly in this genus (Bauchet *et al.*, 2019). As might therefore be expected, more than half of the *V. sativa* genome assembly was comprised of repetitive sequences (Table 3). The assembly contained up to 54,083 contig sequences and included 513 k gaps occupying >500 Mb (Table 2). The short-read technology employed for sequencing might therefore be insufficient to span the repeats. Although construction of contiguous sequences from the short reads was challenging, a near complete gene set was successfully identified in the assembly (Table 2). Whereas it was impossible to compare the genome structure of common vetch with those of relatives due to the fragmented genome sequences, clustering analysis of the gene sequences would provide insights into the gene homoeology in legume species (Figure 4). The genome resources developed in this study will be invaluable for forthcoming gene discovery studies, such as transcriptome analysis and allele mining, in *Vicia*.

We reproducibly observed seven pairs of chromosomes (I to VII) in the root-tip cells of KSR5 (Figure 2), among of which one pair (VII) was so small occupying only 2.7% of the total length of the seven chromosome pairs (Table 1). One type of mini chromosomes, so called B chromosomes which are comprised of repetitive sequence, have been reported in numerous groups of plants so far, but the biological function has not been known (Houben, 2017). B chromosomes are not necessary for the growth and normal development of organisms and show non-Mendelian inheritance patterns (Houben, 2017). This could be one of the reasons for the different chromosome numbers in *Vicia sativa* (Ladizinsky, 1998; Ladizinsky and Waines, 1982; Navratilova *et al.*, 2003). Further chromosome observations and fluorescence in situ hybridization with the repetitive sequences as probes across multiple lines would characterize and identify the mini chromosomes observed in this study. Alternatively, sterility of F1 hybrids derived from crosses between plants with different chromosome numbers should be analyzed to gain insights into the function of the small chromosomes.

Twelve common vetch populations from Japan were examined, each of which contained 2–11 subpopulations (Figure 5, Table 4, Supplementary Figures S4). This suggested that the numbers of founder plants were limited even in populations grown under natural environmental conditions. Heterozygosity is thought to contribute strongly to the survival of plant populations under natural conditions (Canc□ado, 2011). Here, the observed heterozygosity was lower than expected (Table 4), indicating that heterozygosity in common vetch populations was high at the population level but low at the individual level due to self-pollination. This suggested that high heterozygosity at the population level is sufficient to allow adaptation and survival under natural conditions in autogamous common vetch.

Human domestication of wild plant species for agriculture involved selection of individual plants with desirable traits (Izawa *et al.*, 2009; Vaughan *et al.*, 2007). More recently, elite cultivars have been developed with enhanced yield performance to satisfy global food requirements (Hickey *et al.*, 2019). The successive selection of small numbers of individual plants during these processes produced severe bottleneck effects and resulted in decreased genetic diversity and lower tolerance to biotic and abiotic stresses (Canc□ado, 2011). Heterozygosity at specific genome regions was also lost in some wild plants (Figure 6), as reported previously (Mendez-Vigo *et al.*, 2011). This suggested that genome-wide genetic heterogeneity is not necessarily required for plants to survive under natural conditions. Recent studies have proposed *de novo*-, super-, or neo-domestication (Fernie and Yan, 2019; Hickey *et al.*, 2019; Vaughan *et al.*, 2007), whereby genetic loci for agronomically important traits are introduced to cultivated crop varieties from wild plants. However, the high genetic heterozygosity levels from the wild donor plants should be retained during the development of new crops to avoid the bottleneck effects sustained during historic domestication of crop varieties (Litrico and Violle, 2015). Therefore, we propose that new domestication of wild plants should retain high heterozygosity at the population level to capitalize on beneficial traits that increase tolerance to abiotic and biotic stresses, but that agronomically important genetic loci should be fixed to maximize crop potential. The resources generated in this study will provide insights into the *de novo* domestication of wild plants to develop enhanced crop varieties.

Supplementary Data

Supplementary Table S1 Plant materials.

Supplementary Table S2 Genome and transcriptome data.

Supplementary Table S3 Number of KOG functions for protein-encoding genes.

Supplementary Table S4 Number of genes mapped to KEGG pathways.

Supplementary Table S5 Number of GO terms for protein-encoding genes.

Supplementary Figure S1 Cross-validation errors for 12 natural populations of *Vicia sativa* from Japan in admixture analysis.

Supplementary Figure S2 Nucleotide diversity of SNP modules across 12 natural populations of *Vicia sativa* from Japan.

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Data Availability

Sequence data are available from the Sequence Read Archive (DRA) of DNA Data Bank of Japan (DDBJ) under accession numbers DRA004347 for whole genome sequencing, DRA004313 for RNA-Seq, and DRA004301-DRA004312 for ddRAD-Seq (Supplementary Table S2). The DDBJ accession numbers of the assembled sequences are BLWO01000001-BLWO01054083. Genome information is available at Plant GARDEN (https://plantgarden.jp).

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Table 1 Relative chromosome length of *Vicia sativa*, KSR5

Chromosome	Relative length (%)	S.d.*
I	22.3	0.7
II	21.0	0.7
III	18.6	1.3
IV	16.1	1.6
V	10.3	0.7
VI	9.1	0.6
VII	2.7	1.0

*Standard deviation (n = 10)

Table 2 Assembly statistics of the common vetch (*Vicia sativia)* genome assembly VSA_r1.0

	VSA_r1.0
Number of scaffolds	54,083
Assembly size (bp)	1,541,180,487
Scaffold N50 (bp)	90,105
Maximal scaffold (bp)	871,438
Number of gaps	513,235
Gap size (bp)	501,483,283
Complete and single-copy BUSCO	77.5%
Complete and duplicated BUSCO	16.6%
Fragmented BUSCO	2.9%
Missing BUSCO	2.9%
Number of genes predicted	31,146

Table 3 Repeat sequences in the VSA_r1.0 assembly

Repeat type	Length occupied (bp)	%
SINEs[b]	85,029	0.0
LINEs[b]	10,462,622	0.7
LTR elements[b]	200,723,246	13.0
DNA elements	15,595,575	1.0
Helitrons	1,469,970	0.1
Satellites	17,496,670	1.1
Simple repeats	17,496,670	1.1
Low complexity	4,468,370	0.3
Novel repeats	531,016,543	34.5
Total[a]	**782,834,201**	**50.8**

[a] Non-redundant sequence length of the repeats overlapping in the genome.

[b] SINEs: short interspersed nuclear elements; LINEs: long interspersed nuclear elements; and LTR: long terminal repeat.

Table 4 Cluster, heterozygosity, and nucleotide diversity calculated from SNPs of 12 common vetch natural populations in Japan

Population	Sampling location[a]	Number of individuals	Number of SNPs	Number of clusters (K)	Expected heterozygosity (He)	Observed heterozygosity (Ho)	Nucleotide divergency (π)
ABK	Abiko, Chiba, Japan	102	10,040	4	0.313	0.189	0.314
FKO	Fukuoka, Japan	97	9,795	7	0.318	0.057	0.319
KGS	Kagoshima, Japan	109	5,189	8	0.330	0.106	0.330
KMT	Kimitsu, Chiba, Japan	95	7,256	9	0.336	0.087	0.336
KSR	Kisarazu, Chiba, Japan	88	6,450	4	0.340	0.111	0.340
KYT	Kyoto, Japan	104	8,974	8	0.339	0.114	0.338
KZS	Kazusa, Chiba, Japan	97	7,243	4	0.334	0.147	0.334
NGT	Niigata, Japan	100	6,658	3	0.247	0.085	0.248
NGY	Nagoya, Aichi, Japan	102	6,891	5	0.335	0.140	0.335
OKY	Okayama, Japan	99	9,649	11	0.337	0.085	0.336
SDI	Sendai, Miyagi, Japan	100	4,709	2	0.264	0.161	0.262
TNS	Tanashi, Tokyo, Japan	150	7,939	10	0.326	0.153	0.325

[a] Geographical positions are indicated in Figure 1 and Supplementary Table S1.

Figure Legends

Figure 1 Sampling locations in Japan.

Three-letter codes indicate sampling locations in Japan: ABK: Abiko, Chiba; FKO: Fukuoka; KGS: Kagoshima; KMT: Kimitsu, Chiba; KSR: Kisarazu, Chiba; KYT: Kyoto; KZS: Kazusa, Chiba; NGT: Niigata; NGY: Nagoya, Aichi; OKY: Okayama; SDI: Sendai, Miyagi; and TNS: Tanashi, Tokyo.

Figure 2 Chromosomes of the common vetch KSR5.

Roman numerals indicate chromosome pairs, which order is based on chromosome length (I to VII). Bar = 5 μm.

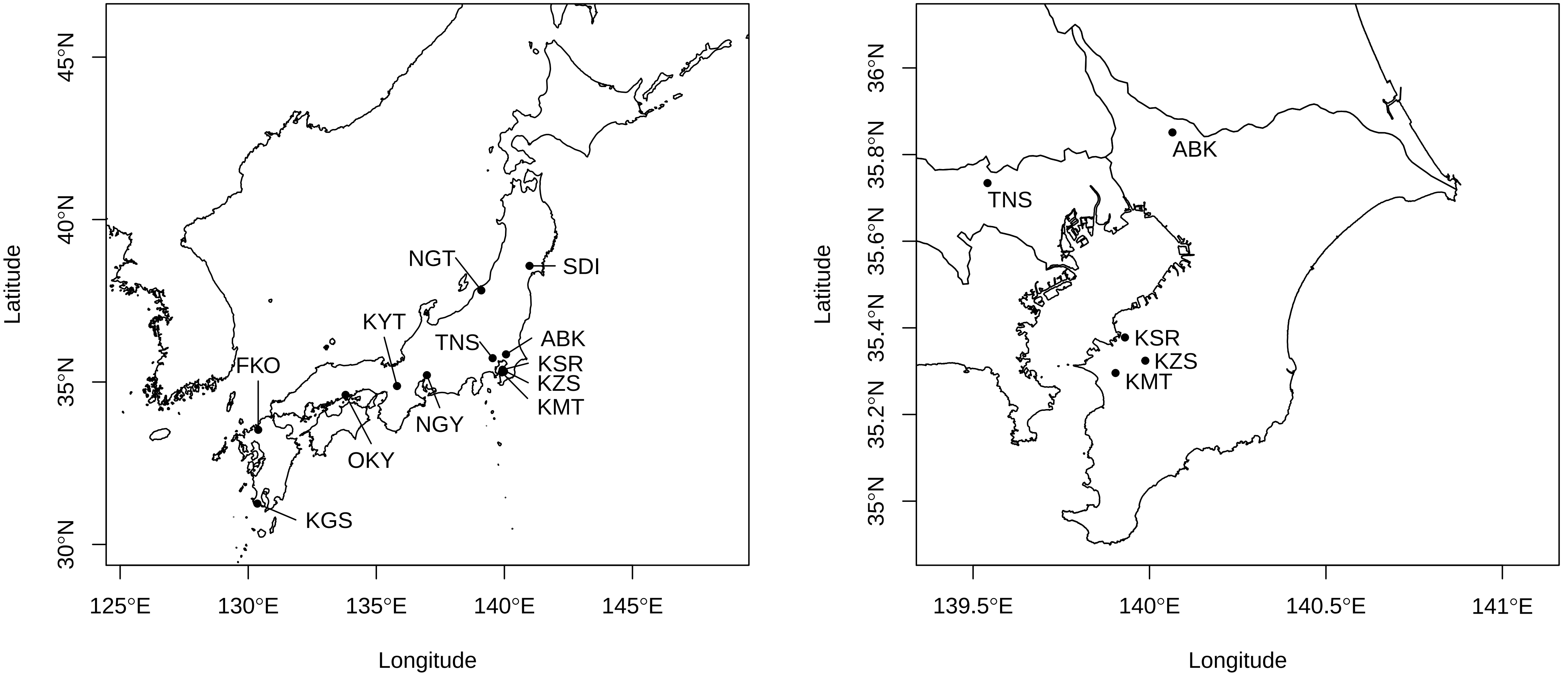
Figure 3 Genome size estimation for *Vicia sativa* with the distribution of the number of distinct *k*-mers (*k*=17) with the given multiplicity values.

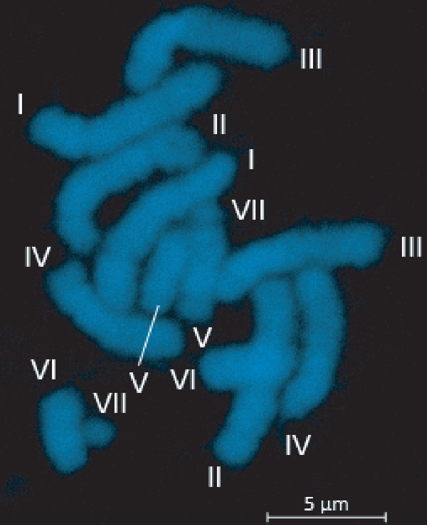
Figure 4 Venn diagram showing numbers of gene clusters in *Vicia sativa* and four additional Fabaceae species.

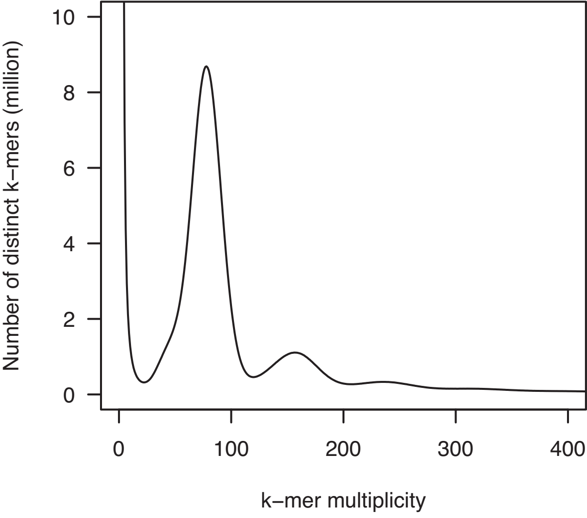
Figure 5 Principal component analysis of 12 natural populations of *Vicia sativa* from Japan.

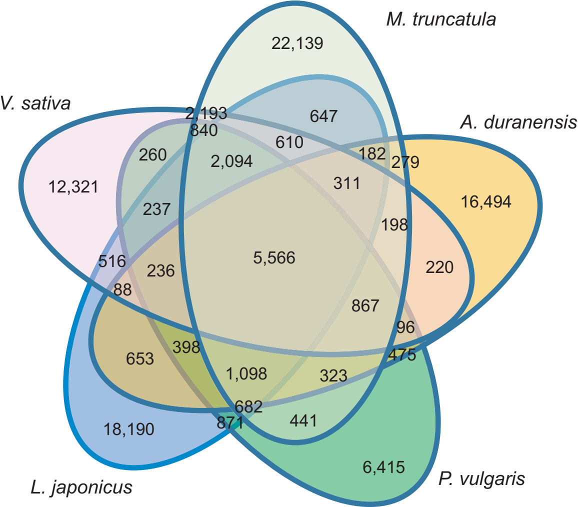
Figure 6 Nucleotide diversity (π) of the SNP module ‘cyan’ (n=190) across 12 natural populations of *Vicia sativa* in Japan.

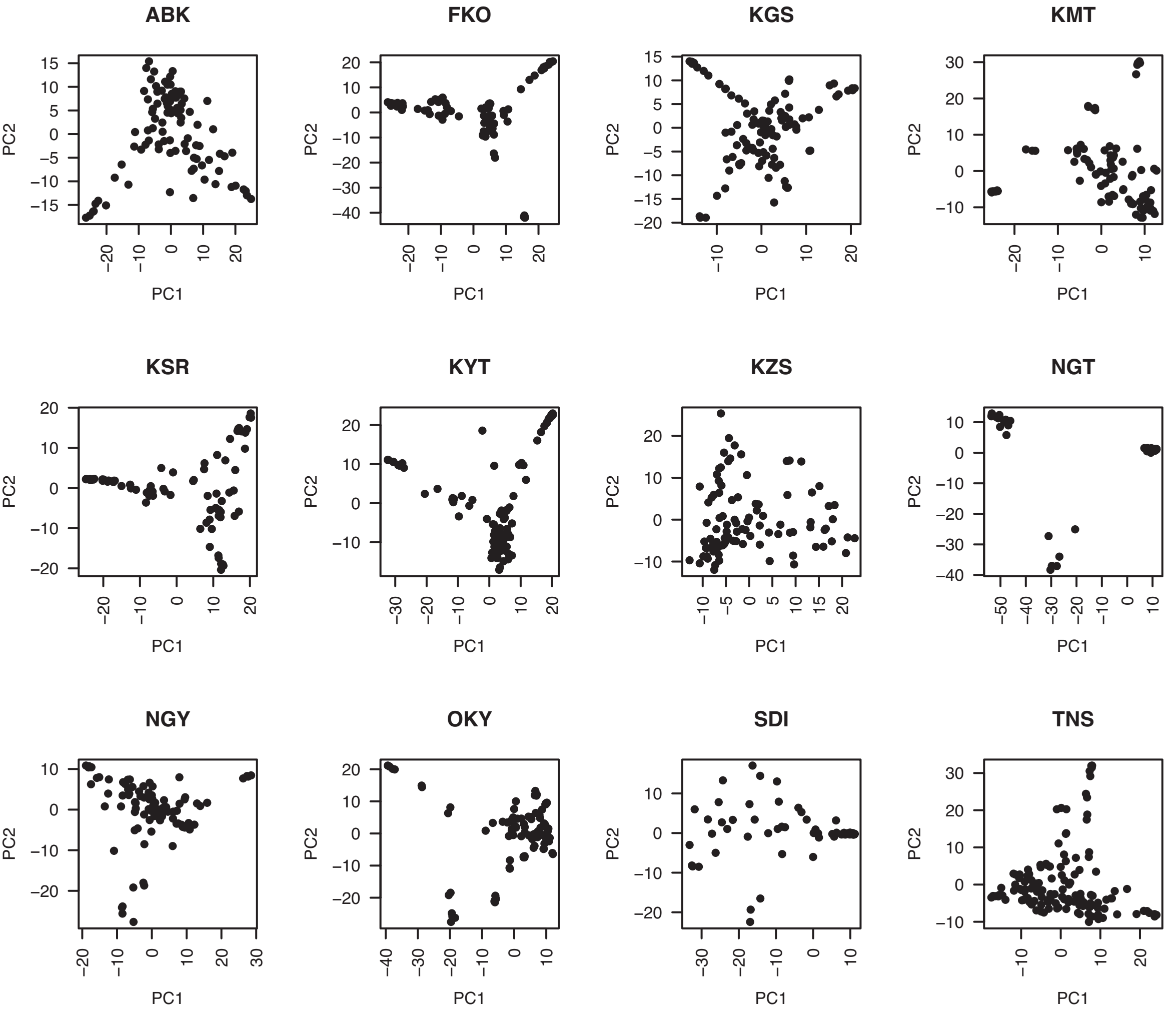
Three-letter codes indicate sampling locations in Japan: ABK: Abiko, Chiba; FKO: Fukuoka; KGS: Kagoshima; KMT: Kimitsu, Chiba; KSR: Kisarazu, Chiba; KYT: Kyoto; KZS: Kazusa, Chiba; NGT: Niigata; NGY: Nagoya, Aichi; OKY: Okayama; SDI: Sendai, Miyagi; and TNS: Tanashi, Tokyo.

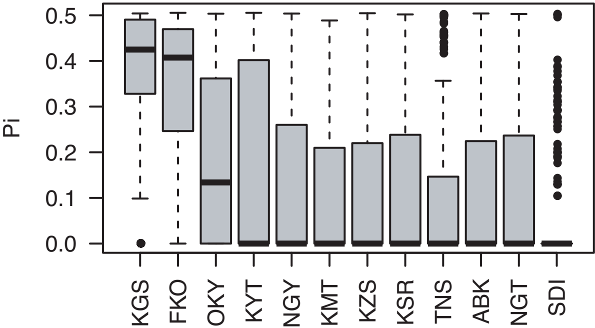

NGT
SDI
KYT
TNS
ABK
KSR
KZS
KMT
FKO
NGY
OKY
KGS
45°N
40°N
35°N
30°N
125°E
130°E
135°E
140°E
145°E
Latitude
Longitude
ABK
TNS
KSR
KZS
KMT
36°N
35.8°N
35.6°N
35.4°N
35.2°N
35°N
139.5°E
140°E
140.5°E
141°E
Latitude
Longitude


III
I
II
I
VII
IV
III
V
VI
V
VI
VII
IV
II
5 µm


Number of distinct k−mers (million)
10
8
6
4
2
0
0
100
200
300
400
k−mer multiplicity


M. truncatula
22,139
V. sativa
2,193
647
A. duranensis
840
260
610
182
279
2,094
12,321
311
16,494
237
198
5,566
516
236
220
88
867
96
653
398
475
1,098
323
682
18,190
871
441
6,415
L. japonicus
P. vulgaris


ABK
FKO
KGS
KMT
KSR
KYT
KZS
NGT
NGY
OKY
SDI
TNS
PC1
PC2
15
10
5
0
−5
−10
−15
−20
−10
0
10
20
30
−30
−40
−50


Pi
0.5
0.4
0.3
0.2
0.1
0.0
KGS
FKO
OKY
KYT
NGY
KMT
KZS
KSR
TNS
ABK
NGT
SDI