

Genetic Architecture Underlying Nascent Speciation—The Evolution of Eurasian Pigs under Domestication

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Abstract

Speciation is a process whereby the evolution of reproductive barriers leads to isolated species. Although many studies have addressed large-effect genetic footprints in the advanced stages of speciation, the genetics of reproductive isolation in nascent stage of speciation remains unclear. Here, we show that pig domestication offers an interesting model for studying the early stages of speciation in great details. Pig breeds have not evolved the large X-effect of hybrid incompatibility commonly observed between “good species.” Instead, deleterious epistatic interactions among multiple autosomal loci are common. These weak Dobzhansky–Muller incompatibilities confer partial hybrid inviability with sex biases in crosses between European and East Asian domestic pigs. The genomic incompatibility is enriched in pathways for angiogenesis, androgen receptor signaling and immunity, with an observation of many highly differentiated cis-regulatory variants. Our study suggests that partial hybrid inviability caused by pervasive but weak interactions among autosomal loci may be a hallmark of nascent speciation in mammals.

Key words: pig domestication, nascent speciation, partial hybrid inviability, Dobzhansky–Muller incompatibility, autosomal interactions.

Introduction

In *On the Origin of Species*, Charles Darwin saw important evolutionary parallels between the process of artificial selection and natural selection, and considered domestication as a valuable entry in exploring mechanisms of speciation (Darwin 1859). The domestication has driven the evolution of domestic animals and resulted in breeds with tremendous phenotypic difference, while their wild progenitors are much less variable. The outcome of domestication process is in accordance with the evolution of new species, a process well known for an accumulation of genetic divergence and reproductive isolations between populations. Given the short domestication history of variety of animals (Wang et al. 2014a; Zhang et al. 2020), the evolution of domesticates can offer a good opportunity to study speciation in nascent stages.

In speciation, the evolution of reproductive isolations is an intriguing process (Coyne and Orr 2004), which has been

intensively explored especially since the observation in 1922 by Haldane, that described heterogametic sex as the sex if one of the two sexes is rare, inviable or sterile in hybrids of inter-specific hybridization (Haldane 1922), a phenomenon widely accepted as Haldane’s rule. This rule has been widely tested in a variety of species (Delph and Demuth 2016), and it was proposed a composite nature of hybrid inviability and hybrid sterility, with hybrid sterility evolving much faster than hybrid inviability in a statistical profiling (Wu and Davis 1993). A hot topic on speciation is about the genetic mechanism underlying the evolutionary process. The prevalence of Haldane’s rule suggests that sex chromosomes play a key role in reproductive barriers despite some exceptions (Moran et al. 2017). In the XY sex determination system, a disproportionately large impact of the X chromosome is widely observed in a variety of species (Coyne 1992; Coyne and Orr 1989), a rule generalized as large X-effect. Further characterization of the

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reproductive isolations have led to identification of many speciation genes that are responsible for either hybrid male sterility (Coyne and Charlesworth 1986; Ting et al. 1998; Phadnis and Orr 2009) or hybrid male inviability (Barbash et al. 2003; Brideau et al. 2006; Phadnis et al. 2015). Deleterious epistatic interaction between speciation genes usually underlies the evolution of reproductive isolations, proposed as Dobzhansky–Muller incompatibility (DMI) speciation model (Dobzhansky 1936; Muller 1942). For example, in a cross between *Drosophila melanogaster* females and *D. simulans* males, a combining effect in hybrid male inviability is observed in an epistatic interaction between *Hmr* (Barbash et al. 2003), *Lhr* (Brideau et al. 2006), and *gzf* (Phadnis et al. 2015).

From a perspective of speciation continuum, an unresolved question is about how two reproductively isolated species evolve from closely related populations. It was well studied that the evolved reproductive barriers differ in sizes of their effect. For example, in *Drosophila*, many species crosses show large effects in causing complete hybrid male inviability or hybrid male sterility (Wu and Davis 1993). Comparably, in mammals, hybrids of two European house mouse subspecies (*Mus m. musculus* and *Mus m. domesticus*) show traits of reduced fertility but incomplete sterility (Turner et al. 2012) of which multiple DMIs were identified (Turner and Harr 2014) and a speciation gene, *Prdm9*, was identified to rescue hybrid male fertility (Mihola et al. 2009). In red flour beetle, the cross of two geographically isolated populations revealed an involvement of X: autosomal interactions both in hybrid incompatibilities obeying Haldane's rule and in hybrid incompatibilities not obeying Haldane's rule (Demuth and Wade 2007). These observations imply that the evolution of reproductive isolation involves the dynamics of DMI evolution during different stages of speciation. To fully understand the speciation process, further studies on speciation in its earliest form, such as nascent speciation is required.

The domestication of pigs offers a good opportunity to explore the nascent speciation. The European and East Asian pigs were independently domesticated from local wild boars (*Sus scrofa*) (Larson et al. 2005; Wu et al. 2007), which colonized the Eurasian continents and split into two geographical populations about 1 million years ago (Groenen et al. 2012; Frantz et al. 2013). Although the Eurasian wild boars may represent different subspecies of *Sus scrofa*, the discriminating difference between them is quite small (Groves 1981; Ruvinsky et al. 2011). Genomic comparison between the Eurasian wild boars showed that there are only 1.27 million fixed differences, amounting to about 0.045% of the pig reference genome (Groenen et al. 2012). Domestication has driven the fast evolution of difference between the Eurasian pig breeds. The Eurasian domestic pigs formed two distinct phylogenetic clades (Larson et al. 2005; Wu et al. 2007). Strong artificial selection has driven the evolution of commercial lines of European pigs with lean growth and elongated body length (Van Laere et al. 2003; Rubin et al. 2012), and many East Asian domestic pigs with extraordinary fat deposition and reproductive performance (Zhang 1986). Despite these differences, hybridization between Eurasian pigs is

common and their hybrids have not shown any evident hybrid incompatibility in swine production. Instead, Eurasian pigs have been frequently used to develop new hybrid breeds or experimental populations for production advantages regarding growth rate or reproductive efficiency (Zhang 1986; Guo et al. 2009; Li et al. 2009b). In addition, gene flow between the Eurasian domestic pigs and subsequent human-mediated selection were observed (Bosse et al. 2014; Frantz et al. 2015; Chen et al. 2020), indicating that the reproductive barriers, if there are any, are weak. Here we show that the Eurasian domestic pigs can offer as an ideal model for studying nascent speciation, where weak DMIs start to evolve in part of Eurasian pig genome. Using the pig model, deep insights can be achieved into evolutionary steps of nascent speciation.

Results

To study the putative reproductive barriers evolved between Eurasian domestic pigs, we constructed a F2 population ($n=589$) using a cross between five Large White (European) boars and sixteen Min (East Asian) sows (Wang et al. 2014b). The Large White and Min pigs are well known for their large difference in productive performance, for example growth rate (mean daily weight gain: 939 ± 2.00 g for Large White and 507 ± 2.00 g for Min pig) and fat deposition (backfat thickness: 10.3 ± 0.25 mm for Large White at 100 kg and 37.3 ± 1.6 mm for Min pig at 65 kg) (China National Commission of Animal Genetic Resources 2011). The F2 offspring consists of 298 males and 291 females, without any evident bias toward sexes. We performed whole genome resequencing of the F0 founder populations (30-fold depth for each individual), and all F0, F1 (nine males and 45 females), and F2 individuals were genotyped using Porcine SNP60 BeadChips (Illumina, USA) containing 62,163 SNPs according to the manufacturer protocol. The resequencing and chip data allowed us to trace the Large White and Min pig genomes inherited by F2 males/females and to detect reproductive barriers resulted from a mismatch between the genomes of different origins.

Genomic Pattern of Biased Inheritance in F2 Population

The haplotypes of autosomes were inferred using the pedigree as [supplementary information](#) to correct genotyping and recombination switch errors by applying a duoHMM method (O'Connell et al. 2014). The haplotypes of X chromosomes were determined by supplying a pseudopedigree in which paternal X chromosome of F2 females was assumed to inherit directly from those of paternal grandma, and maternal grandpa to be a homozygote on chromosome X for both F2 male and female ([supplementary fig. S1, Supplementary Material](#) online). Recombination and F0 genomic sequence inheritance by F2 were further determined by inhouse programs. This strategy successfully identified the European or East Asian origin of genomic sequences inherited by F2 with a considerable low error rate ($<0.05\%$) estimated from the difference observed between raw genotypes and the inferred

inheritance data (supplementary table S1, Supplementary Material online).

Haplotype analysis revealed that the F0 genomes were not inherited uniformly by F2 individuals. Using the Large White allele frequency (p_{LW}) in 1-Mb genomic windows as a measure of inheritance deviation, the F2 showed an average p_{LW} of 0.50 together with a strong bias in regional genomes. An enrichment of Large White genomic sequence in F2 was observed on chromosomes 3, 15, and 16, in comparison to an enrichment of Min genomic sequence on chromosomes 6 and 17, and all these regions showed a clustering distribution on chromosomes (fig. 1a; supplementary table S2, Supplementary Material online). Only maternal X chromosomes in F2 were used in the analysis since the paternal X chromosome in F2 females was always from Min pig (F0 females). Different from autosomes, the maternal X chromosome did not show strong bias of p_{LW} . The highest p_{LW} is 0.55 observed at 3-Mb window spanning from 52 Mb to 55 Mb of chromosome 3, and the lowest is 0.46 at 65 Mb–66 Mb of chromosome 17.

The regional p_{LW} bias may have two putative causes. First, the p_{LW} bias could be caused by strong linkage between chromosomal fragments from genomic regions of recombination deserts. Second, the p_{LW} bias was possibly associated with genomic islands of speciation that are highly differentiated between the F0 founder populations and causing hybrid incompatibility in the F2 populations. To determine the F1 meiotic recombination inherited by F2 individuals, the chromosomal crossovers inherited in F2 genomes were screened. A total of 12,986 autosomal recombination breakpoints were identified, including 9,742 from paternal and 12,986 from maternal genomes. In the maternal X chromosome, 743 recombination breakpoints were identified. Mapping of the recombination breakpoints shows that the p_{LW} bias is less likely to associate with recombination deserts. The largest two chromosomes (1 and 13) contain long recombination deserts in the chromosomal centers (supplementary fig. S2, Supplementary Material online), where the p_{LW} did not deviate remarkably from an expectation of 0.5. Comparatively, the population differentiation (F_{ST}) in genomic windows with

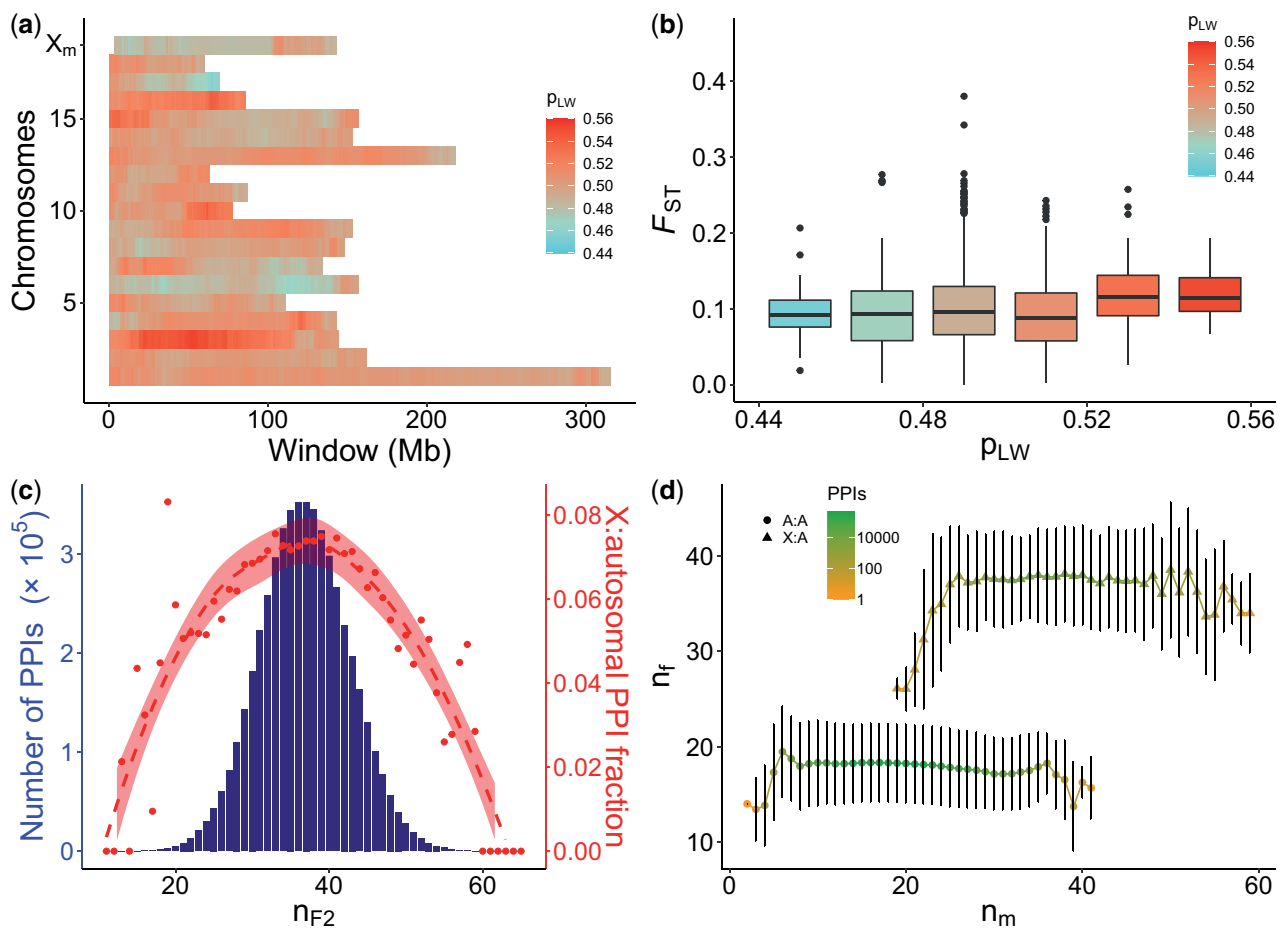


FIG. 1. Genomic feature of biased inheritance in F2 and F2 distribution in protein–protein interactions (PPIs). (a) Distribution of Large White allele in F2 genomes. The frequency of Large White allele (p_{LW}) is drawn in 1-Mb nonoverlapping sliding windows on chromosomes 1–18, and maternal X chromosome. (b) Difference in population differentiation (F_{ST}) in windows with varying level of p_{LW} in F0 founder populations. (c) F2 sample distribution in interchromosomal PPIs grouped by n_{F2} values and the fractions of X:autosomal PPI in the PPI groups. (d) Weak sexual antagonism of interautosomal PPIs and absence of sexual antagonism in X:autosomal PPIs. The sexual antagonism of PPIs was measured by the number of F2 males (n_m) and of F2 females (n_f). The result is plotted as the mean and standard deviation of n_m grouped by PPIs with same n_f values. A: A indicates the interautosomal PPIs, and X: A indicates the interactions between maternal X chromosome and autosomes.

$p_{LW} > 0.53$ (mean $F_{ST} = 0.12$) was significantly higher than those with $p_{LW} < 0.53$ (mean $F_{ST} = 0.10$) (t test; $P = 3.76 \times 10^{-6}$) (fig. 1b). The result indicates that the p_{LW} bias is likely to reflect genomic islands of speciation between the Large White and Min founder populations.

Multiple Interautosomal DMIs on Partial Hybrid Male Inviability

To test whether reproductive barriers evolved between Eurasian domestic pigs, we focused on protein–protein interactions (PPIs) and tested their putative roles as DMIs in the F2 genomes. The pig PPIs were predicted by mapping of human orthologous proteins in BioGrid database (Oughtred et al. 2019). In this analysis, the PPI network was investigated from genomic interactions between two loci that encode relevant proteins. To explore the different effects, the PPIs with varying genotype combinations were considered as different PPIs. As a consequence, a total of 5,480,672 distinct PPIs were identified, including 5,162,992 interchromosomal and 317,680 intrachromosomal PPIs (supplementary table S3, Supplementary Material online).

We focus on the PPI effect on the hybrid inviability of the F2 experimental population, and the number of F2 individuals in each PPI was calculated. The intrachromosomal PPIs were not considered since the grouping of F2 individuals into PPIs was largely affected by linkage between loci on same chromosomes. For 5,162,992 interchromosomal PPIs, the number of F2 individuals (n_{F2}) was observed with a mean of 36.80 on each PPI and a standard deviation of 5.81 (fig. 1c). We failed to find any interchromosomal PPIs that were completely lacking the presence of F2 males, of F2 female, or of both, indicating the absence of interchromosomal PPIs with large effect in forming DMIs of full hybrid inviability. Despite the absence of full hybrid inviability, possibility still exists of partial hybrid inviability effects in the pig cross, inferring from the n_{F2} variation. The X: autosomal PPIs ($n = 361,120$) consist of 6.99% of the interchromosomal PPIs, and it is unexpected to observe X: autosomal PPIs with relative low occurrence in interchromosomal PPI groups at high and low ends of n_{F2} values (fig. 1c). X: autosomal PPIs account for 3.05% of interchromosomal PPIs in groups with $n_{F2} \leq 15$ and 5.48% in groups with $n_{F2} \leq 25$. Since the low n_{F2} values may be associated with partial hybrid inviability, it implies that the X: autosomal PPIs may have less influence in mediating partial hybrid inviability in the cross of Eurasian pigs.

Despite an absence of PPIs for full hybrid inviability, the interautosomal and X: autosomal PPIs showed a difference of their potential in conferring sex-biased partial hybrid inviability. We observed a pattern of weak sexual antagonism of interautosomal PPIs with the majority (99.44%) showing a statistically significant negative correlation between the numbers of F2 males (n_m) and F2 females (n_f) ($r = -0.82$ for $6 \leq n_m \leq 31$; $P = 2.32 \times 10^{-7}$) (fig. 1d). The sexual antagonism indicates an effect on partial hybrid inviability commenced its evolution as asymmetric in the two sexes for most interautosomal PPIs. For interautosomal PPIs with $n_m \leq 5$, the individual numbers in the two sexes showed a trend

that the partial inviability of both sexes were affected in a similar direction although did not attain statistical significance ($r = 0.74$; $P = 0.26$). A possible explanation is that the interautosomal interactions consisted of at least two groups of PPIs, and majority has a weak antagonistic role in conferring partial hybrid inviability between the two sexes. For X: autosomal PPIs, the X-linked interactor genotypes were not directly comparable between two sexes, since the F2 females always inherited paternal X chromosomes that are eventually from the paternal grandma (Min pig). After assuming that hemizygous X-linked interactors with Min alleles in F2 males have effects equivalent to X-linked interactors homozygous for Min alleles in F2 females, no statistically significant negative correlation was observed between two sexes ($r = -0.10$; $P = 0.65$) for most (94.42%) of the considered X: autosomal PPIs with $26 \leq n_m \leq 48$, despite its positive correlation in those with $n_m < 26$ ($r = 0.98$; $P = 9.68 \times 10^{-5}$) (fig. 1d). These observations imply that interautosomal PPIs in the Eurasian pig cross, rather than the X: autosomal PPIs, may have increased capacity and flexibility to mediate the hybrid male/female ratio by having evolved with sex-biased DMI effects.

To examine the putative role of interchromosomal PPIs as sex-biased DMIs conforming to Haldane's rule, PPIs with the same n_{F2} value were classified into one group and the sex ratio was computed for PPI groups. We tested the association of F2 male ratio and PPI groups with varying n_{F2} values. Overall, the F2 male ratio remains a level approximating to 0.506 for most of the PPI groups. Remarkably, F2 male ratios ranged from 0.31 to 0.49 in a subset of interautosomal PPI groups with $n_{F2} \leq 15$ (fig. 2a), an observation implied the presence of partial hybrid male inviability for these PPI groups. Although the total number of interautosomal PPIs ($n = 350$) in PPI groups with low F2 male ratios was limited, they may represent a small subset of strongest interautosomal DMIs between the European and East Asian pig genomes. Interestingly, the F2 male ratio variation was much less prominent when the X: autosomal interactions were considered. Similar to the analysis of effect of sexual antagonism, we assumed that hemizygous X-linked interactors with Min alleles in F2 males have effects equivalent to X-linked interactors homozygous for Min alleles in F2 females. Therefore, the mean n_{F2} of the considered X: autosomal PPI groups is expected to be 2-fold of the mean n_{F2} of interautosomal PPI groups. We discovered that the F2 male ratio variation was much less prominent among groups of the considered X: autosomal PPIs, and the lowest F2 male ratio was 0.45 (fig. 2a). In sharp contrast to lower F2 male ratios mediated by interautosomal PPI in groups with $n_{F2} \leq 15$, X: autosomal PPIs were absent in most of interchromosomal PPI groups with $n_{F2} \leq 15$ (fig. 1c). The higher potential of interautosomal interactions in shaping the much lower F2 male ratios was consistent with their larger roles in evolving sex-biased DMIs. Combining the observation in figure 1c, the result suggests that large X-effect was not dominant in the Eurasian pig cross, unveiling a profound role of autosomes in specifying partial hybrid male inviability in nascent speciation.

The observation of low hybrid male ratios in low- n_{F2} PPI groups implies that these low- n_{F2} PPIs may have affect hybrid

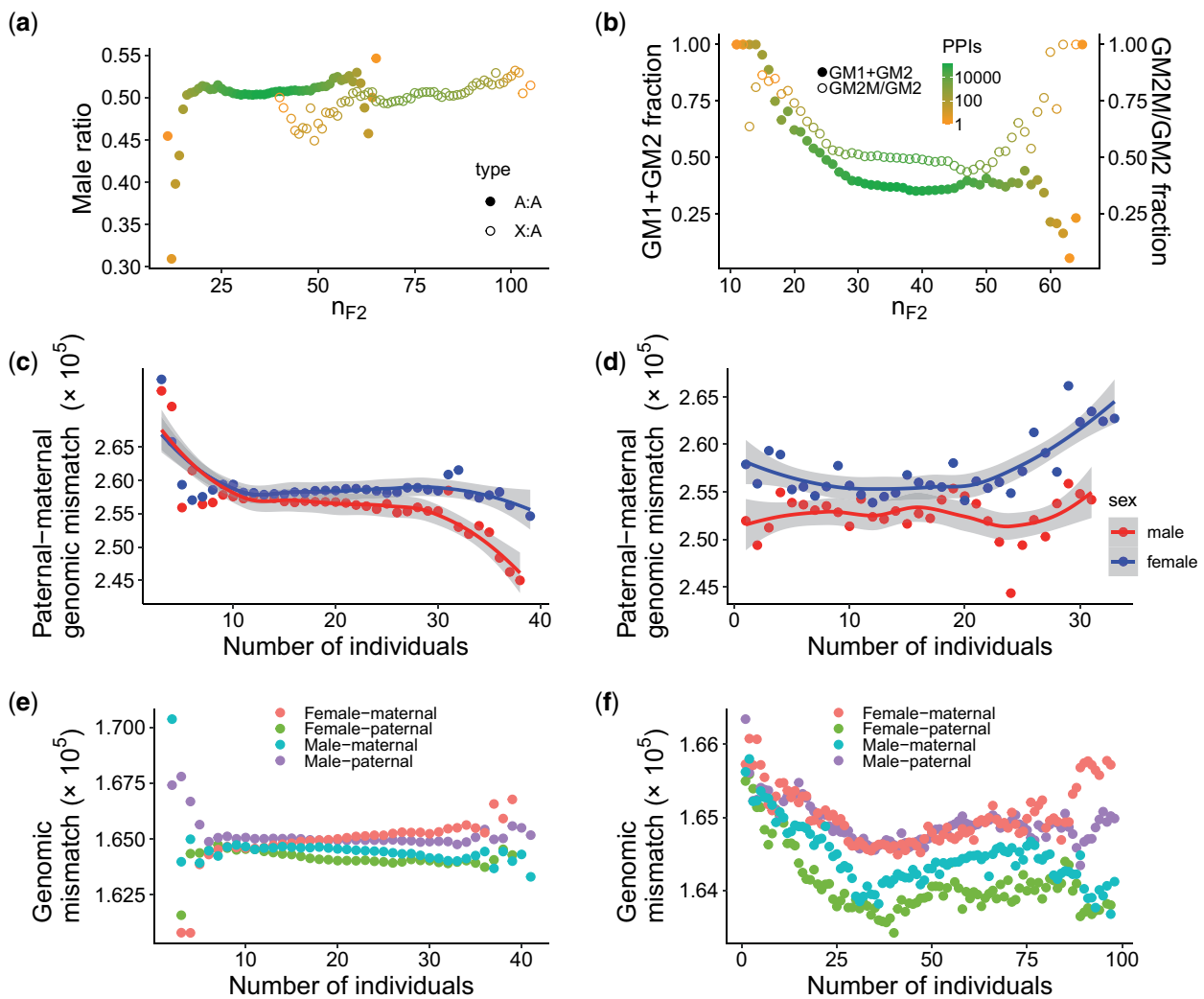


FIG. 2. Dobzhansky–Muller incompatibilities (DMIs), hybrid inviability, hybrid male inviability, and sex difference in tolerance to DMIs in paternal and maternal genomes. (a) Low hybrid male ratio observed in interautosomal PPIs, but not in X: autosomal interactions. The male ratio was calculated for PPIs grouped by n_{F2} values. The color is indicating the number of PPIs used in the statistics. A: A indicates interautosomal PPIs, and X: A indicates X: autosomal PPIs. (b) Negative correlation between n_{F2} and the fraction of Large White and Min pig genomic mismatches (GM1+GM2) in PPI groups with low n_{F2} values (≤ 30). The GM2M/GM2 ratio indicates the fraction of genomic mismatches in GM2 with the Large White allele inherited from maternal genome at the second locus of PPI. The color is indicating the number of PPIs used in the analysis. (c) Sex difference in tolerance of interautosomal PPI genomic mismatch between paternal and maternal genomes. (d) Sex difference in tolerance of intra-autosomal PPI genomic mismatch between paternal and maternal genomes. Interautosomal genomic mismatch (e) and intra-autosomal genomic mismatch (f) in the paternal and maternal genomes of F2 individuals classified by cross-sex or same-sex inheritance.

inviability of both sexes but have larger effect on F2 males. To test this, we compared the n_f distributions in two groups of PPIs ($n_{F2} \leq 15$ and $n_{F2} > 15$). We found that the n_f (mean = 7.94, standard deviation is 1.57) in the $n_{F2} \leq 15$ PPI groups was significantly lower than that (mean = 18.18, standard deviation is 4.14) in the $n_{F2} > 15$ PPI groups (t test; $P < 2.2 \times 10^{-16}$). This indicates that these groups of PPI causing hybrid male inviability are also having DMI effects causing hybrid female inviability.

Evolutionary Tendency of Hybrid Inviability and Sex Difference in DMI Tolerance

An intriguing question is to unravel the reason that partial hybrid male inviability, rather than partial hybrid female inviability, evolves as a consequence of multiple and weak-effect

interautosomal DMIs in partial hybrid inviability without an observation of evident large X-effect. To further explore this, we profiled interautosomal PPIs in the Eurasian pig genomes with effective incompatibilities and characterized the evolutionary tendency of interautosomal DMIs of partial hybrid inviability toward partial hybrid male inviability.

To follow the dynamics of interautosomal PPIs evolution into effective DMIs of partial hybrid inviability, we analyzed genotype constitutions of interautosomal PPI groups with varying n_{F2} values. If many interautosomal PPIs form effective DMIs between the Large White and Min pig genomes, low- n_{F2} PPI groups should be associated with high level of genotype mismatches between two loci of interautosomal PPIs. Our analysis confirmed this speculation and showed that Large White and Min pig genomes accumulated substantial

incompatible interautosomal PPIs, of which the genotype mismatch may have caused partial hybrid inviability of F2 individuals. Interautosomal PPIs with low n_{F2} were enriched between two loci with first being homozygous for the Min allele while the second being homozygous for Large White allele (genotype mismatch 1; GM1) or being heterozygous (genotype mismatch 2; GM2). A strong negative correlation ($r = -0.97$; $P = 5.91 \times 10^{-13}$) was identified between n_{F2} and the fraction of GM1+GM2 interactions in PPI groups when n_{F2} less than or equal to 30 (fig. 2b). Totally, the PPI groups ($n_{F2} \leq 30$) contain 659,466 interautosomal PPIs, accounting for 13.73% of the total interautosomal PPIs ($n = 4,801,872$). A total of 287,380 GM1+GM2 interactions were observed in the PPI groups ($n_{F2} \leq 30$), accounting for 43.58% of interactions in these PPI groups, and the percentage (53.82%, 64,457 GM1+GM2 interactions) is much more significant when PPI groups with $n_{F2} \leq 25$ were considered. In comparison, the GM1+GM2 fractions showed a small variation and remained at a low level when $n_{F2} > 30$, approximating to 0.375 in expectation (6 out of 16 PPI combinations). The high GM1+GM2 fraction variation in interautosomal PPIs with $n_{F2} \geq 60$ were mainly attributed to the low number of GM1+GM2 interactions ($n = 94$ in total). The negative correlation between GM1+GM2 ratio and n_{F2} in a wide range at low end ($n_{F2} \leq 30$) reflected the partial hybrid inviability evolved from substantial GM1+GM2 mismatched interactions in Large White and Min autosomes. It is important to note that the low F2 male ratios were only observed in PPIs with $n_{F2} \leq 15$ (fig. 2a), the high fraction of GM1+GM2 interactions in PPI groups with $n_{F2} \leq 30$ indicated that the majority of these PPI groups ($n_{F2} \leq 30$) have led to partial hybrid inviability roughly symmetrical in both males and females. In the cases of heterozygous at the second locus of GM2, the Large White allele can inherit either from paternal (GM2P) or maternal (GM2M) genomes. We found that, in a range of $n_{F2} \leq 30$, the GM2M ratio in GM2 is increasing steadily when the n_{F2} decreases ($r = -0.90$, $P = 7.29 \times 10^{-8}$) (fig. 2b). This observation indicated the GM2 DMIs have mainly resulted from epistatic interactions between Large White and Min autosomal alleles in maternal genome. It was unexpected to find that the GM2M ratio in GM2 increases with n_{F2} when n_{F2} is greater than 50 ($r = 0.68$, $P = 9.57 \times 10^{-6}$). Further analysis showed that this GM2M increase is associated with simultaneous decreases of both GM2P and GM2M in the total PPI groups when n_{F2} is greater than 50, while the GM2P decreases more significantly than GM2M.

It is unclear why hybrid male inviability evolves faster than hybrid female inviability given with the DMI effect on hybrid inviability evolved from substantial GM1+GM2 autosomal interactions in both sexes. Given low F2 male ratios in PPI groups with $n_{F2} \leq 15$ and partial hybrid inviability in PPI groups with $n_{F2} \leq 30$, it seems that the partial hybrid male inviability may have evolved as an extreme form of partial hybrid inviability, reaching a state of which GM1+GM2 PPIs have broken the two-sex symmetry and thus have caused larger DMI effect in F2 males. To test this hypothesis, a comparison was conducted on two sexes regarding their tolerance

to interautosomal DMIs between their paternal and maternal genomes. We focused on the number of interautosomal PPIs genotype mismatched (genomic mismatch) within paternal and within maternal genomes, but the genomic mismatch was not compensated between paternal and maternal genomes in fertilization. After calculating the retained interautosomal PPI genomic mismatches between paternal and maternal genomes of F2 (see Materials and Methods), we found F2 females with higher tolerance of interautosomal PPI genomic mismatches than F2 males (Wilcoxon rank-sum test; $P = 1.01 \times 10^{-8}$) (fig. 2c). This trend was further observed in intra-autosomal PPIs ($P = 3.03 \times 10^{-13}$) (fig. 2d), despite the strong linkage among loci within a chromosome. The lower tolerance to interautosomal PPI genomic mismatches between paternal and maternal genomes in F2 males may have resulted in genome-wide explanation for the arrival priority of partial hybrid male inviability over partial hybrid female inviability even before the large X-effect evolves dominantly effective. The results support an evolutionary tendency of partial hybrid inviability toward partial hybrid male inviability.

Interestingly, we found that the higher tolerance of genomic mismatches between paternal and maternal genomes in F2 females could not be explained simply by maternal effect. An analysis of the genomic mismatches within either paternal or maternal genomes showed that the F2 females did not always tolerate high levels of genomic mismatches. We found that maternal genomes of F2 females tolerated much higher level of genomic mismatch than paternal genomes of F2 females either in the interautosomal PPIs (Wilcoxon rank-sum test; $P < 3.33 \times 10^{-11}$) (fig. 2e) or in the intra-autosomal PPIs ($P < 2.20 \times 10^{-16}$) (fig. 2f). Similarly, the F2 males had higher genomic mismatch tolerance in their paternal genomes than in their maternal genomes, either in the interautosomal PPIs ($P < 2.20 \times 10^{-16}$) (fig. 2e) or in the intra-autosomal PPIs ($P < 2.20 \times 10^{-16}$) (fig. 2f). The paternal genomes of F2 males could tolerate genomic mismatch higher than the paternal genome of F2 females either in interautosomal ($P < 2.20 \times 10^{-16}$) or in intra-autosomal PPIs ($P < 2.20 \times 10^{-16}$). This seems that the cross-sex inheritance of F2 from F1 results in a low level of tolerance of genomic mismatch in the inherited genomes.

Association of p_{LW} Bias on Chromosome 3 with DMIs

To further confirm the p_{LW} bias in F2 genomes represented islands of speciation, an analysis was conducted to examine their overlapping with genomic regions of PPIs with low n_{F2} . Using a threshold of GM1+GM2 ratio ≥ 0.6 (equivalent to $n_{F2} \leq 17$), a total of 1,668 interchromosomal PPIs were identified. These PPIs mainly consisted of interactions between chromosome 3 and other autosomes (fig. 3a). Of the 1,668 PPIs, 1,252 were related to chromosome 3, accounting for about 75.06% of the interchromosomal interactions. In a comparison, only 34 X: autosomal interactions were observed, accounting for about 2.04% of them. On chromosome 3, for 1-Mb sliding windows overlapping with PPIs of $n_{F2} \leq 17$, the p_{LW} was significantly higher (mean $p_{LW} = 0.54$; standard deviation is 0.01) than that in sliding windows not overlapping

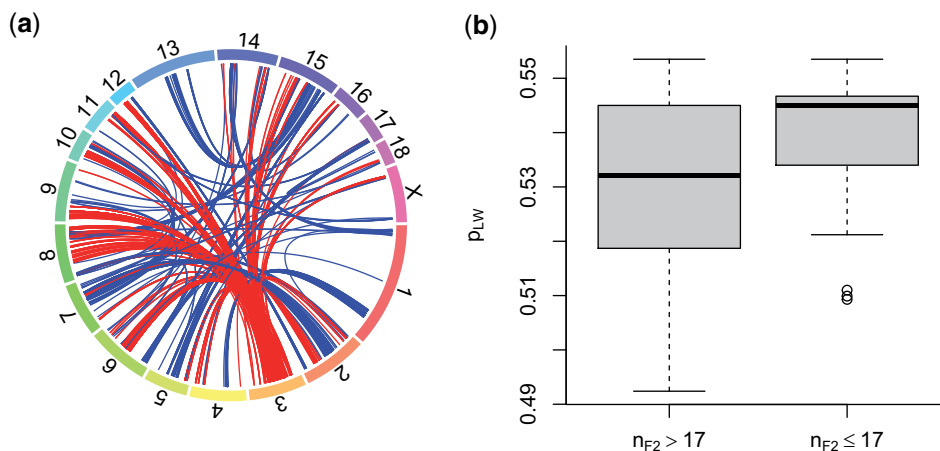


FIG. 3. Chromosomal distribution of DMIs and their association with p_{LW} bias. (a) The genome-wide distribution of DMIs for PPI groups with GM1+GM2 ratio ≥ 0.6 . The PPIs between chromosome 3 and other autosomes are plot in red. (b) The p_{LW} difference between genomic regions on chromosome 3 containing PPIs for different n_{F2} groups.

(mean $p_{LW} = 0.53$; standard deviation is 0.02) (t-test; $P = 1.45 \times 10^{-7}$) (fig. 3b). This indicated that the p_{LW} bias in chromosome 3 results from negative selection against deleterious epistatic interactions between chromosome 3 and other autosomes.

Genes and Genomic Variants in the DMIs

We turned to identify the genes and pathways associated with DMIs between the Large White and Min pigs. To reduce the noise from near neutral PPIs, we focused on PPIs in groups with GM1+GM2 ratio threshold greater than 0.6. Pathway analysis showed that these PPIs were enriched in several biological pathways, including “VEGFA-VEGFR2 signaling pathway” (WP: WP3888; adjusted $P = 6.45 \times 10^{-4}$), “androgen receptor signaling pathway” (WP: WP138; adjusted $P = 1.30 \times 10^{-2}$) and pathways regarding immune system (supplementary table S4, Supplementary Material online). It is well known that VEGFA signaling through VEGFR2 is the major pathway activating angiogenesis important for vascular development in embryos (Abhinand et al. 2016; Shalaby et al. 1995). The incompatibility in angiogenesis may have resulted from growth rate mismatch between an outstanding performance in the Large White and ordinary performance in the Min pig (Zhang 1986). The androgen receptor signaling pathway is important for sexual development in both males and females (Chang et al. 2013; Larkins et al. 2016), and the incompatibility in it may have affected hybrid inviability or hybrid male inviability during the embryo development.

We conducted a genomic variant analysis despite the difficulty in further tests of their biological roles in conferring hybrid inviability given with their weak effects. Screening of genomic variants revealed substantial highly differentiated variants were presented in noncoding sequences at genomic regions encoding interactors in these incompatible PPI groups (GM1+GM2 ratio ≥ 0.6). With a stringent criterion of SNP differentiation ($F_{ST} \geq 0.80$) between the Large White and Min founder pigs, 22,162 autosomal SNPs were identified. After implementing cis-regulatory sequence prediction in the pig genome using data from ENCODE project as a reference

(Davis et al. 2018; Ma et al. 2020), we found 2,667 autosomal SNPs located inside putative cis-regulatory sequences with counterparts in human genomes annotated as DNase I hypersensitive sites, histone chemical modification sites, transcription factor binding sequences, or transcription factor recognizing DNA motifs (supplementary table S5, Supplementary Material online). In contrasts, only 48 missense mutations from 37 protein coding genes were identified (supplementary table S6, Supplementary Material online). Among the 2,667 putative cis-regulatory variants, 222 were located in cross-species conserved noncoding sequence (CNS). Further characterization of the 222 variants in CNS identified eight variants in transcription factor recognizing DNA motifs (supplementary table S7, Supplementary Material online). The abundance of highly differentiated cis-regulatory variants supports the hypothesis that part of the evolved DMIs results from mismatched gene expressions. Further functional assays are required to validate the function of genes and variants in the reproductive barriers.

Discussion

In this study, we found the pig domestication can provide a good model to explore the evolution of reproductive barriers and genetic architectures in nascent speciation. In colonization of wild progenitors in the Eurasian continents and subsequent domestications, reproductive barriers have evolved between the Eurasian domestic pigs. Though the Haldane’s rule is not evident in the cross of many Eurasian domestic pigs (Zhang 1986), our data suggest evolution of multiple Dobzhansky–Muller incompatibilities as weak reproductive barriers in the pig. The distinct observation in domestic pigs may help to fill the knowledge gap relevant to the process of evolution from moderately differentiated populations to reproductively isolated species.

The evidence from our study in pigs supports an unusual role of autosomes on hybrid inviability in nascent speciation, compared to large role of X chromosome in advanced stages of speciation (Coyne 1992; Coyne and Orr 1989; Coyne and

Orr 2004; Presgraves 2008) and in population level (Demuth and Wade 2007; Lachance and True 2010). It is of a particular interest to compare the role of autosomes and the role of X chromosome in the process of speciation. The contradicting results of autosomes' and X chromosome's role may reflect observations on different stages of speciation. It is well known that the X chromosome evolves much faster than autosomes in speciation, referring a process of faster-X evolution (Coyne and Orr 1989; Coyne and Orr 2004; Presgraves 2008, 2018). In nascent speciation, lack of effective large X-effect on hybrid inviability in the pig cross is possibly associated with a low level of genomic differentiation between the Eurasian pigs, and in contrast the autosomes may have more effects in specifying partial hybrid inviability given a higher probability to evolve weak DMIs from a higher genomic capacity of PPIs. This raises an interesting question about the race between autosomes and X chromosome to cause hybrid incompatibility in the sequence of speciation. In addition, our observation of the nascent speciation of pigs, the interautosomal PPIs showed a steady sexual antagonism on hybrid inviability, while X: autosomal PPIs did not. The data supports a dominant role of autosomes in conferring the early form of sex-biased incompatible effects. Combining our results with earlier observations (Coyne 1992; Coyne and Orr 1989), we propose a hypothetical model of role switching from autosomes to X chromosome along with the sequence of speciation, in which the hybrid incompatibilities specified by autosomes initiate the nascent speciation while hybrid incompatibilities specified by X chromosome evolve more and more efficient and dominate the former. Our study implies that the hybrid incompatibility caused by pervasive but weak interactions among autosomal loci may be a hallmark of nascent speciation in mammals. Further tests using data from other domestic animals, such as dogs, may provide more details about this model.

We unraveled that partial hybrid inviability and partial hybrid male inviability can evolve quite early in speciation. This phenomenon seems contradictory to the observation of faster evolution of hybrid sterility than hybrid inviability in *Drosophila* and mammals (Wu and Davis 1993), but may have plausible explanations. In this study, our focus was not on the hybrid sterility and the relative rate between hybrid sterility and hybrid inviability is not available in the pig cross. Another difference is that the hybrid inviability phenotype in this experimental pig population is weak and partial, while strong and full hybrid inviability phenotype was used in the statistics for generalization (Wu and Davis 1993). It is possible that reproductive barriers on full hybrid sterility tend to have large effects, and reproductive barriers on partial hybrid inviability can evolve very early in nascent speciation but with minor effect. This is consistent with the hypothetical genetic difference responsible for reproductive barriers evolved in nascent and advanced stages of speciation. Partial hybrid inviability could be common in the cross of the Eurasian domestic pigs, according to the high fractions of GM1+GM2 interactions in PPI groups from the lower-end of n_{F_2} . We propose that the partial hybrid male inviability could be an evolutionary end of the partial hybrid inviability when a subset of DMIs

have evolved relatively large effects on hybrid inviability and have broken their effect symmetry between two sexes. Despite the wide distribution of DMIs for partial hybrid inviability in pig genome, it will be of a huge challenge to characterizing their biological significance in a litter due to their weak effects. Testing the effect in large-scale populations could be a powerful approach in studying the nascent reproductive barriers.

Materials and Methods

This study was approved by the Animal Care and Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences. The care and treatment of the pigs comply with the guidelines of animal use protocols approved by the Animal Care and Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Science, China (Approval ID: SMKX-20191213-01).

Experimental Population

A three-generation (F0, F1, and F2) resource population was constructed by intercrossing Large White boars and Min sows (Wang et al. 2014b). In F0 generation, four Large White boars were mated with 16 Min sows. Nine boars and 46 sows from the F1 population were intercrossed to produce an F2 population consisted of 589 adult animals. All the animals were raised under the same nutritional and environmental conditions, and were housed at the experiment base of Institute of Animal Science, CAAS.

Genome Sequencing and SNP Calling

Genomic DNA was extracted from ear tissues following a standard phenol-chloroform method. The paired-end genomic reads of F0 samples were sequenced at 30-fold depth using the HiSeq2000 platform with libraries of 500-bp inserts. All the samples were used for genotyping with the Illumina Porcine SNP60 BeadChips (Illumina, USA). The genomic reads of F0 individuals were mapped the Duroc reference genome (Groenen et al. 2012) using the Burrows-Wheeler Aligner (BWA) (Li and Durbin 2009). SAMtools (Li et al. 2009a) was used to sorting, merging and removing potential PCR duplications. Genome Analysis Toolkit (GATK) was used for SNP calling for each F0 sample (McKenna et al. 2010).

Haplotype Inference and Analysis of Inheritance

The Porcine SNP60 chip data was used to infer the haplotype of all individuals in the experimental population. The haplotypes of autosomes were inferred using SHAPEIT by supplying pedigree information to correct genotyping errors and recombination switch errors (O'Connell et al. 2014). For X chromosomes, different strategies were used in haplotype inference. We constructed two pseudopedigrees, and one for F2 males and one for F2 females (supplementary fig. S1, Supplementary Material online). In brief, the X chromosome of maternal grandpa (F0) was assumed to be a homozygote in its full length, and the paternal X chromosomes in F2 females were considered inheriting directly from their paternal grandma (F0). These assumptions made the inference of X chromosome haplotypes feasible and the accuracy was high by applying the pseudopedigree information to error

correction. The haplotypes of X chromosome were inferred with a similar pipeline in autosomal haplotype inference but supplying the pseudopedigree information.

The F2 inheritance of F0 genomic fragments were analyzed with inhouse R scripts that estimated the overall difference between the inferred F2 haplotypes and F1 haplotypes and then determined the F1 meiotic recombination breakpoints inherited in F2 genomes. The inheritance of F2 genomes from F1 genomes were then determined according to the recombination breakpoints. The inheritance analysis was also performed to determine the F1 inheritance of F0 genomes. Eventually, the origin of F2 genomes from F0 genomes was determined by combining the inheritance map across the three generations. To analyze the robustness of the obtained inheritance information for regional genome, we compared the genotypes of SNPs in the inferred F2 haplotypes and the genotypes in the F0 data (both the raw data and the inferred haplotypes), and error rates were estimated as the fraction of SNPs with data inconsistency.

To determine the inheritance bias of F0 genome in the F2 genomes, the Large White allele frequency (p_{LW}) in 1-Mb nonoverlapping windows were calculated. For autosomes, the p_{LW} was averaged on both F2 paternal and F2 maternal genomes. For X chromosome, the p_{LW} was calculated on the maternal X chromosome, since the paternal X chromosome in F2 females was always from the Min pig.

Population Differentiation Analysis

F_{ST} (Weir and Cockerham estimator) was calculated on 1-Mb nonoverlapping windows between the Large White and Min founder populations, using the VCFtools software (Danecek et al. 2011).

Protein–Protein Interaction Analysis

The protein–protein interactions (PPIs) in pigs were derived from a mapping of the annotated human protein counterparts in BioGrid database (Oughtred et al. 2019) to the pig genome. The pig PPIs were classified as interchromosomal and intrachromosomal according to the protein-encoding gene distribution on chromosomes. Interautosomal and X: autosomal PPIs were further classified as interchromosomal PPIs.

For two loci encoding a PPI, assuming to be A and B loci, the genotypes of the two loci were used to determine the types of PPI. Suppose that the Large White allele is denoted as A (or B) and Min as a (or b), there are four types of each locus contributing to PPI types. For example, for the locus A, there are four types, including (denoted as paternal/maternal) A/A, A/a, a/A, and a/a. Therefore, for each A: B pair, 16 (4×4) interautosomal PPIs were classified according to genotypes at the two loci and paternal/maternal origins of alleles. Only eight X: autosomal PPIs were classified on each pair of loci for each sex. The X: autosomal PPIs in F2 males and F2 females were considered as different PPIs, and therefore a total of 16 X: autosomal PPIs were finally identified for each pair of A:B loci.

Hybrid Inviability and Hybrid Male Inviability Analysis

The numbers of F2 (n_{F2}), F2 male (n_m), and F2 female (n_f) were calculated to indicate the effects on hybrid inviability and hybrid male inviability for each of the PPIs. The PPIs were classified into different groups according to their n_{F2} , n_m , and n_f values. Hybrid male inviability was measured as hybrid male ratio in population level but not from an observation from an individual level. The hybrid male ratio was calculated as the ratio of F2 males to the number of F2 individuals in PPI groups classified by n_{F2} . The hybrid inviability was investigated from the correlation between the n_{F2} and the fraction of genomic mismatches of genes in PPI groups. The genomic mismatches were considered as PPIs with first locus being homozygous for the Min allele, while the second being homozygous for Large White allele (GM1) or being heterozygous (GM2). The overall hybrid inviability was estimated by the correlation between n_{F2} and fraction (GM1+GM2) was tested when the PPI groups have small n_{F2} values.

During the analysis of sexual antagonism of PPIs between males and females, we divided the PPIs into interautosomal and X: autosomal PPIs. In the analysis of sexual antagonism for interautosomal PPIs, the PPIs were grouped with n_m , and then the mean and standard deviation of numbers of F2 females (n_f) in each PPI group were calculated. Noted that for each interautosomal PPI, the n_{F2} is equal to the sum of n_m and n_f . In the analysis of sexual antagonism for X: autosomal PPIs, similar calculation was conducted but restricted to X: autosomal PPIs with maternal X-linked interactor carrying the Min allele. Noted that for each of the considered X: autosomal PPI, the n_m and n_f values are at a level comparable to n_{F2} of interautosomal PPIs.

Tolerance of Genomic Mismatch between Large White and Min Pig Alleles

To investigate the sex difference in the tolerance of genomic mismatches, the interactors in PPIs were identified with one from Large White allele and the other from Min allele, both in paternal and in maternal genomes. The tolerance to genomic mismatch in F2 males and F2 females was measured as the mean number of mismatched PPIs in their paternal and maternal genomes that were retained and not compensated between their paternal and maternal genomes, in different groups of PPI classified by varying number of male and female individuals. The analysis was conducted for both interautosomal and intra-autosomal PPIs. The numbers of mismatched PPIs were also analyzed within either the paternal or the maternal genomes, considering the same-sex and cross-sex inheritance of the paternal/maternal genomes of the F2 individuals.

Gene Enrichment and Genomic Variant Analysis

Genes from PPI groups with GM1+GM2 ratio ≥ 0.6 were used for gene enrichment analysis in pathways. The pathways were identified using the gProfiler software (Reimand et al. 2011). The genomic variants in the PPI groups (GM1+GM2 ratio ≥ 0.6) were identified in the F0 founder populations. We selected SNPs with $F_{ST} \geq 0.80$ for further analysis. The annotation of SNPs was conducted using the OrthReg software

that implements a mapping of human cis-regulatory sequences into the pig genome (Ma et al. 2020).

Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online.

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Author Contributions

H-B.X., Y-P.Z., L-X.W., and Z-B.Z. conceived the project. H-B.X., C-Y.F., and X.Y. conducted the data analysis. H-B.X. and C-Y.F. wrote the article. A.C.A. improved the manuscript. L-G.W., L-C.Z., and L-X.W. constructed the experimental population.

Accession Numbers

The newly generated genome-wide sequence data of Large White and Min pigs (FASTQ format) in this study have been deposited in the Genome sequence Archive in Big Data Center (<http://gsa.big.ac.cn/>; accession project number: PRJCA004200).

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