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Development of selective markers linked to a major QTL for parthenocarpy in eggplant (*Solanum melongena* L.)

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Abstract Parthenocarpy, the ability to set fruits without pollination, is a useful trait for setting fruit under unfavorable conditions. To identify the loci controlling parthenocarpy in eggplant (*Solanum melongena* L.), we constructed linkage maps by using co-dominant simple sequence repeat and single nucleotide polymorphism markers in F_2 populations derived from intraspecific crosses between two non-parthenocarpic lines (LS1934 and Nakate-Shinkuro) and a parthenocarpic line (AE-P03). Total map distances were 1,414.6 cM (ALF2: LS1934 × AE-P03) and 1,153.8 cM (NAF2: Nakate-Shinkuro × AE-P03), respectively. Quantitative trait locus (QTL) analyses revealed two QTLs on chromosomes 3 and 8, which we denoted as *Controlling parthenocarpy3.1* (*Cop3.1*) and *Cop8.1*, respectively. The percentage of phenotypic variance explained (PVE) of *Cop3.1* was 6.3% in ALF2 (LOD = 4.2) and 10.6% in NAF2 (LOD = 3.0). The PVE of *Cop8.1* was 45.7% in ALF2 (LOD = 23.8) and 29.7% in NAF2 (LOD = 7.9). Using a population of backcross inbred lines, we confirmed the effect of *Cop8.1*, but there was no evidence to support the contribution of *Cop3.1*. We need to verify the effect of *Cop3.1* under various temperature conditions. In addition, we clarified the effectiveness of selective SSR markers, emf21H22 and emh11J10, mapped on each side of *Cop8.1* in other F_2

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populations derived from various parental combinations. This is the first report concerning QTL analysis of parthenocarpy in eggplant using molecular markers. It will be useful in marker-assisted selection and in revealing the genomic mechanism underlying parthenocarpy in eggplant.

Introduction

In normal fruit development, the initiation of fruit set depends on successful pollination and fertilization (Gillaspy et al. 1993). After pollination, the level of the endogenous auxin indole-3-acetic acid is increased in eggplant (Lee et al. 1997), and it is well known that synthetic auxins such as 4-chlorophenoxyacetic acid stimulate the growth of unpollinated ovaries. Focusing on this finding, Rotino et al. (1997) developed transgenic eggplants with the *iaaM* gene from *Pseudomonas syringae* that exhibited increased auxin biosynthesis in their cells and organs (Gaudin et al. 1994). In the transgenic eggplants, fruit set without fertilization, and the fruits developed parthenocarpically from emasculated flowers (Acciarri et al. 2002; Rotino et al. 1997).

Genetic studies of parthenocarpy, particularly in tomato, have identified eight parthenocarpic genes—*pat* (Beraldi et al. 2004; Mazzucato et al. 1998), *pat-2* (Fos et al. 2000, 2003), *pat-3/pat-4* (Fos et al. 2001; Gorguet et al. 2008), *pat4.1/pat5.1*, and *pat4.2/pat9.1* (Gorguet et al. 2008)—with potential applications in the production of parthenocarpic fruits. Five of these sources—*pat*, *pat4.1*, *pat4.2*, *pat5.1*, and *pat9.1*—have been mapped on genetic linkage maps (Beraldi et al. 2004; Gorguet et al. 2008). Using these maps, attempts at map-based cloning have been made, and parthenocarpy-causing genes may soon be isolated. However, each parthenocarpy-causing gene identified in tomato has problems associated with its use, such as pollinated flowers producing

a very low seed set (Mazzucato et al. 1998), genetic background controlling the level of parthenocarpy (Fos et al. 2000), different sizes of seeded and seedless fruits being set (Fos et al. 2001; Gorguet et al. 2008), and decreased parthenocarpy at low temperature (Gorguet et al. 2008).

Genetic analysis of parthenocarpy in eggplant, which belongs to the same genus, *Solanum*, as tomato, began in 1994 at the NARO Institute of Vegetable and Tea Science (NIVTS; Mie, Japan) with the crossing of a European parthenocarpic cultivar, Talina, and a Japanese non-parthenocarpic cultivar, EPL1 (Yoshida et al. 1998). Segregation tests in F_2 and BC_1F_1 populations suggested that parthenocarpy was controlled by a single major gene (Yoshida et al. 1998). In a subsequent experiment with a different cross combination—a European parthenocarpic cultivar, Mileda, by a Japanese non-parthenocarpic line, ASL-1—progeny testing confirmed the existence of a dominant single major gene (Kuno and Yabe 2005). Doganlar et al. (2002) reported a detailed interspecific linkage map of eggplant compared with tomato, and using this linkage map, Frary et al. (2003) performed advanced quantitative trait locus (QTL) analyses of morphological traits in eggplant. Recently, Barchi et al. (2010) reported an intraspecific linkage map. Most of the above-mentioned markers, developed by Doganlar et al. (2002) and Barchi et al. (2010), were RFLP (restriction-fragment-length polymorphism) and AFLP (amplified-fragment-length polymorphism) markers, respectively. Therefore, it is difficult to apply those markers to detailed genetic studies on other populations of different intraspecific crosses, because of the low numbers of polymorphisms. To solve this problem, Nunome et al. (2003a, b, 2009) developed simple sequence repeat (SSR) markers for *S. melongena* species, and Fukuoka et al. (submitted) developed single nucleotide polymorphism (SNP) markers derived from expressed sequence tag (EST) sequences to fill in the gaps and complete the detailed integrated linkage map.

We report here the construction of an intraspecific map of F_2 populations (*S. melongena* × *S. melongena*) using the above marker resources, which enabled us to analyze the complex trait of parthenocarpy. We evaluated this trait as a quantitative character. The purpose of this study was to confirm the feasibility of marker-assisted selection with the developed markers and to discuss the prospect for studies of the mechanisms of parthenocarpy in eggplant.

Materials and methods

Plant materials

For genetic analyses, we developed two populations of F_2 plants derived from the crosses between two non-parthenocarpic eggplant lines, LS1934 and Nakate-Shinkuro, with a parthenocarpic line, AE-P03, which we denoted ALF2 (LS1934 × AE-P03) and NAF2 (Nakate-Shinkuro × AE-P03). LS1934 was collected in Malaysia (Sakata et al. 1996), Nakate-Shinkuro is a Japanese cultivar, and AE-P03 is a Japanese line developed at NIVTS from F_9 plants derived from the cross between Nakate-Shinkuro and the European parthenocarpic cultivar Talina (Kikuchi et al. 2008).

Plant growth conditions and evaluation of the level of parthenocarpy

Emasculated, unfertilized flowers of AE-P03 set normal parthenocarpic fruits at a high rate, while those of LS1934 and Nakate-Shinkuro set malformed fruits (Fig. 1a–c). Kikuchi et al. (2008) noted that cultivation at a low temperature reduces the number of dropped flowers, thereby allowing more accurate evaluation of parthenocarpy level. So we grew the F_2 plants in the winter–spring of 2004–2005 (ALF2: $n = 135$) and 2007–2008 (NAF2: $n = 93$) in a warming greenhouse at a minimum air temperature of 15°C, and natural lighting, having sown the seeds in September 2004 and 2007, respectively. One to two months after sowing, ALF2 seedlings were transplanted into 21-cm-diameter plastic pots, and NAF2 seedlings were transplanted into the ground. A few days before anthesis, 5–10 flowers were emasculated, and 2 months later we measured the length of all 5–10 fruits and checked for the absence of seeds.

Fruits at least as long as the maximum length of the emasculated non-parthenocarpic parents (LS1934, 60 mm; Nakate-Shinkuro, 70 mm) were scored as normal, and those shorter were scored as malformed. If a cavity was observed inside the fruit, the fruit was evaluated as malformed, even if it was longer than the standard.

We calculated the level of parthenocarpy (L_p) as the ratio of the number of normal fruit set (N_{normal}) to that of the total fruit set (N_{total} = number of emasculated flowers except dropped flowers):

$$L_p = N_{normal}/N_{total} \times 100. \tag{1}$$

SSR analysis

Total DNA was isolated from frozen leaves by using a DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). Total of 1,054 SSR markers developed by Nunome et al. (2009) were screened for detecting polymorphisms among four eggplant lines, AE-P03, LS1934, Nakate-Shinkuro and WCGR112-8 (introduced from India). Following these data, we chose the markers which were polymorphic among as many combinations as possible, and labeled with fluorescent pigment (FAM/VIC/NED/PET). Among the
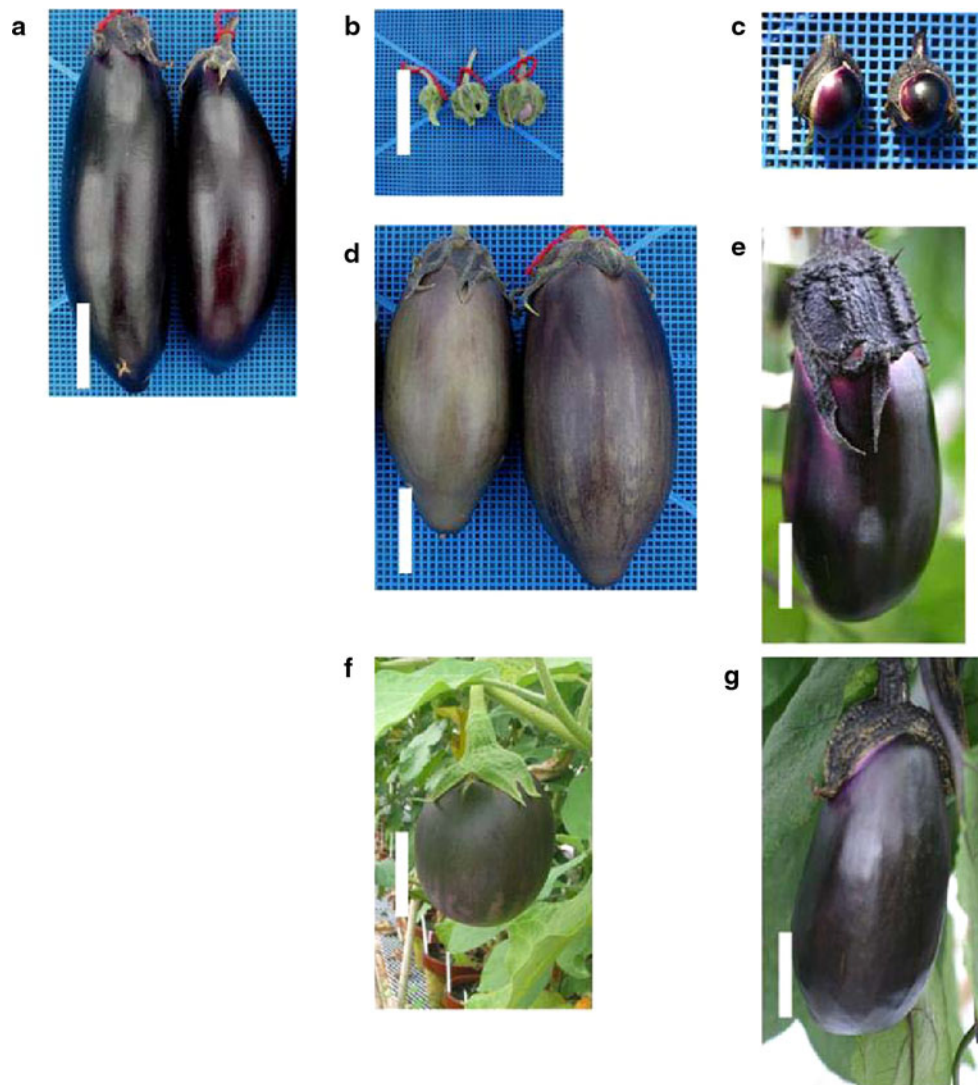
Fig. 1 Shape of emasculated fruits: **a** AE-P03, **b** LS1934, **c** Nakate-Shinkuro, **d** ALF1 (LS1934 × AE-P03), **e** NAF1 (Nakate-Shinkuro × AE-P03). Shape of pollinated seeded fruits: **f** LS1934, **g** Nakate-Shinkuro. *Scale bar* indicates 50 mm

labeled markers (total of 453 markers), we screened for usable markers for mapping in the ALF2/NAF2 populations (ESM_1). PCR was carried out in a volume of 8 µl containing 10 ng genomic DNA, 200 µM dNTPs, 200 nM each primer, 1× reaction buffer (Roche Diagnostics GmbH, Mannheim, Germany), and 0.25 U Taq DNA polymerase (Roche). Reaction conditions were as follows: an initial 3 min at 94°C; 10 cycles of 30 s at 94°C, 1 min at 65–56°C (decreasing by 1°C every cycle), 1 min at 72°C; and a final 5 min at 72°C. Fragments amplified with fluorochrome-labeled primers (FAM, VIC, NED and PET) were diluted with 80 times their volume in water, dissolved in formamide, and then analyzed on an automated DNA sequencer (3730 DNA Analyzer; Applied Biosystems, Foster City, CA, USA) with a GeneScan-500LIZ size standard (Applied Biosystems). Fragment length

was determined with GeneMapper software (Applied Biosystems).

SNP analysis

We screened for polymorphic SNP markers designed from the sequences of hypothetical intron regions estimated by the comparison of the tomato genome sequence and the orthologous EST sequence sets of eggplant, tomato, and potato (Fukuoka et al., submitted). All SNPs markers developed by Fukuoka et al. (submitted), total of 630 markers, were screened for detecting polymorphisms among parents of ALF2/NAF2 populations (ESM_1). To genotype these SNPs, we used the Tm-shift genotyping method originally reported by Wang et al. (2005) and partially modified by Fukuoka et al. (2008) to improve detection efficiency.

Map construction

We genotyped 135 ALF2 and 93 NAF2 F_2 plants with the markers. Fukuoka et al. (submitted) created an integrated linkage map of eggplant (LWA2010) derived from LS1934 × AE-P03 ($n = 93$) and LS1934 × WCGR112-8 ($n = 90$). Detailed data of the integrated linkage maps and the molecular markers are available from the Vegmarks database at http://vegmarks.nivot.affrc.go.jp. Among the 954 markers (324 SSR; 630 SNP) mapped on it, we selected suitable markers for the QTL analyses on ALF2 and NAF2 populations, on the basis of map position and the polymorphic data among parents (ESM_1). We used MAPMAKER/EXP 3.0b software (Lander et al. 1987) to determine the linkage groups (LGs) and the order of the markers by Kosambi's mapping function (Kosambi 1943) with a LOD threshold of 3.0 and a map distance of <50 cM. A graphic representation of the LGs was created in MapChart 2.2 software (Voorrips 2002).

QTL analysis

QTL analyses were performed using the levels of parthenocarpy of the F_2 populations and the linkage map from each experiment. Composite interval mapping (CIM) was performed using Windows QTL Cartographer software v. 2.5 (Wang et al. 2005) with the parameter settings of model 6 and forward and backward stepwise regression with a threshold of $P < 0.05$. The genome was scanned at 2-cM intervals. One thousand permutation tests were performed to establish empirical LOD thresholds (ALF2, 3.7; NAF2, 3.6) at the 5% level for experiment-wise Type I errors (Churchill and Doerge 1994). The LOD threshold of 2.5 was used to declare the presence of a QTL, provided that their peaks were mapped at approximately the same positions in both experiments. To confirm the effect of the detected QTLs, we grouped F_2 progeny according to the genotypes of the markers linked to the QTLs, and compared differences among groups with the Tukey–Kramer test.

Verification of the effectiveness of the markers nearest to the parthenocarpy QTLs

A population of 128 backcross inbred lines (BILs) (F_9) was derived by single-seed descent from reciprocal crosses between an F_1 plant (LS1934 × AE-P03) and LS1934. Seeds were sown in September 2010. One to two months after sowing, the seedlings were transplanted into the ground in a warming greenhouse and were grown at an air temperature of 15°C. The level of parthenocarpy was evaluated as above.

BIL plants were genotyped with markers linked to parthenocarpy QTLs detected in the ALF2 population. As in the experiments described, we grouped the BIL plants according to the marker genotypes linked to the QTLs and compared the differences among groups.

We also developed three additional populations of F_2 progeny, crossing the Japanese non-parthenocarpic cultivars Aodaimaru, Kamonasu, and Satsuma-Shironaganasu (all *S. melongena*) with AE-P03, which we denoted as AAF2, KAF2, and SAF2, respectively (ESM_2). The three cultivars originate from different parts of Japan and express wide variations in fruit color and shape (Aodaimaru: green/round; Kamonasu: purple/round; Satsuma-Shironaganasu: light green/long). Seeds were sown in September 2009. Total DNA was isolated from young leaves on Multiscreen-NA and -FB filter plates (Millipore, Billerica, MA, USA) as described by Matsumoto et al. (2005). We screened these F_2 individuals with markers linked to the parthenocarpy QTL (see "Results"), and transplanted them to 21-cm-diameter pots. Three flowers were emasculated for evaluation of the level of parthenocarpy as above. The size standards for evaluating parthenocarpy were as follows: Aodaimaru, 55 mm; Kamonasu, 30 mm; Satsuma-Shironaganasu, 75 mm.

Results

Evaluation of the level of parthenocarpy

All of the emasculated flowers of LS1934 and Nakate-Shinkuro developed into malformed, unseeded fruits (LS1934: $L_p = 0\%$, $n = 28$; Nakate-Shinkuro: $L_p = 0\%$, $n = 18$), which were notably smaller than the pollinated, seeded fruits (Fig. 1b, c, f, g). AE-P03 usually sets normal-sized fruits ($L_p = 94.3\%$; $n = 35$), even without pollination (Fig. 1a). F_1 plants derived from ALF1 (LS1934 × AE-P03) and NAF1 (Nakate-Shinkuro × AE-P03) exhibited partial parthenocarpy, suggesting dominant inheritance of this trait (Fig. 1d, e). The two F_2 populations, ALF2 and NAF2, showed a continuous distribution of the level of parthenocarpy (Fig. 2). Aside from this, results of Shapiro–Wilks test with P values of 5.544e-07 (=ALF2) and 1.264×10^{-4} (=NAF2) suggested that these data did not follow the normal distribution.

Linkage map and map comparison

We used two sets of F_2 populations and constructed linkage maps (ALF2, $n = 135$; NAF2, $n = 93$). After screening the polymorphic markers available for the ALF2 population, we selected 118 SSR markers and 132 SNP markers (ESM_1). The map constructed using these markers consisted of 12 LGs covering a total map distance of 1,414.6 cM (ESM_1). Similarly, we selected 174 markers (SSR, 125; SNP, 49) for the NAF2 population (ESM_1),

Fig. 2 Frequency distribution of level of parthenocarpy in two F₂ populations (*white bar* ALF2, *black bar* NAF2). To compare the different-sized populations, the number of plants in each rank was converted to the ratio of the total number of plants. The mean values of the parents and F₁ plants in the respective populations (*1st* ALF2, *2nd* NAF2) are shown in *parentheses*
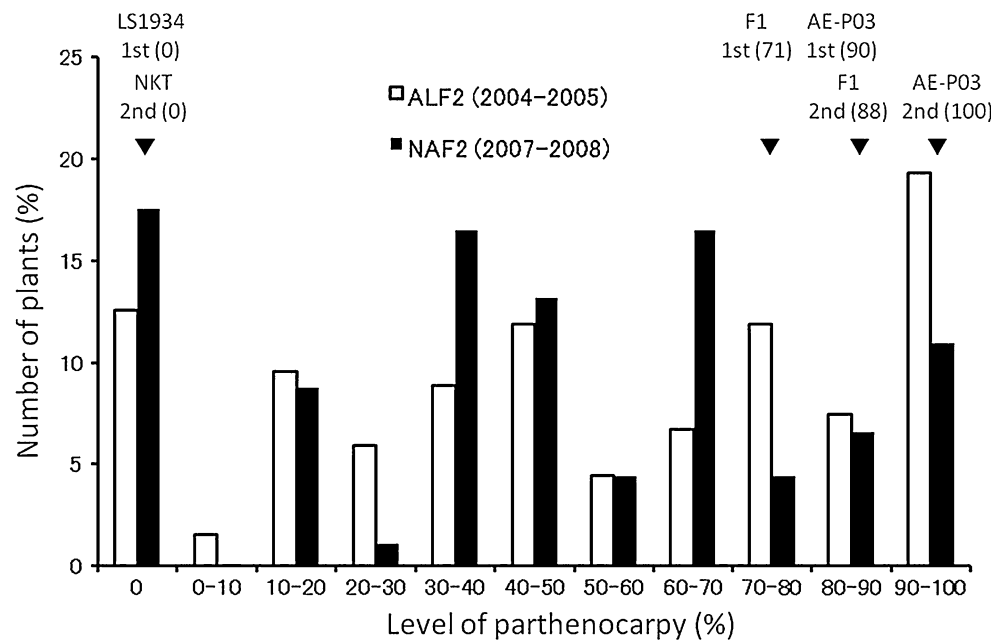
and to cover the region of low marker density on chromosome 3, developed an additional two markers (SOL7068bS, est_smfl18k11S) by sequencing the polymorphic SNPs next to the SNPs mapped on the integrated map (LWA2010; Fukuoka et al., submitted, http://vegmarks.nivot.affrc.go.jp) using the sequencing primer pairs developed for SNP screening. The linkage map constructed using the 176 markers consisted of 15 LGs covering a total map distance of 1,153.8 cM (ESM_1). By comparing the two maps and the integrated linkage map, we assigned all LGs for ALF2 and NAF2 to those of LWA2010 (Fig. 3). However, three gaps on the NAF2 linkage map resulted in 15 non-converged LGs.

QTL analysis

We detected four QTLs with LOD >2.5 in the ALF2 population and three in NAF2 (Fig. 3). Among these QTLs, we detected two that we denoted *Cop3.1* and *Cop8.1* (Fig. 3). Comparison of the eggplant linkage map with that of tomato using the common markers showed that the LGs on which the QTLs were mapped corresponded to chromosomes 8 (*Cop8.1*) and 3 (*Cop3.1*) (Fukuoka et al. submitted, http://vegmarks.nivot.affrc.go.jp; Figs. 4, 5). The major QTL, *Cop8.1*, was detected with LOD values of 23.8 in ALF2 (PVE = 45.7) and 7.9 in NAF2 (PVE = 29.7), which are higher than the empirical threshold values (ALF2, 3.7; NAF2, 3.6) in the same region of chromosome 8 (Fig. 4). The ranges of the LOD peaks above the empirical threshold were approximately 22 cM (ALF2: emf21A23–emh11J10) and 24 cM (NAF2: emf21A23–ecm023) (Fig. 4). The LOD values of *Cop3.1* were 4.2 in

ALF2 (PVE = 6.3) and 3.0 in NAF2 (PVE = 10.6) (Fig. 5). The LOD value of *Cop3.1* in ALF2 was higher than the empirical threshold (3.7), but that of *Cop3.1* in NAF2 was lower than the empirical threshold (3.6) (Table 1; Fig. 5).

We grouped F₂ progeny according to the marker genotypes nearest to *Cop3.1* and *Cop8.1* and calculated the mean values of the levels of parthenocarpy (Table 2). The F₂ progeny with non-parthenocarpic parental homozygous genotypes at the markers nearest to *Cop3.1* and *Cop8.1* expressed a notably lower level of parthenocarpy (ALF2, 3.7%; NAF2, 16.7%; Table 2). The F₂ progeny with AE-P03 homozygous genotypes at the same loci expressed a higher level of parthenocarpy (ALF2, 81.0%; NAF2, 75.0%; Table 2). *Cop8.1* had a major effect on parthenocarpy. *Cop3.1* had a minor effect, especially when the genotype of the nearest marker to *Cop8.1* was not homozygous with that of the parthenocarpic parent. This result, however, was not statistically significant (Table 2). We genotyped 128 BIL plants with two markers (est_cpa03j24 and est_ls502j22) nearest to the parthenocarpy QTLs detected in the ALF2 population (Table 1), and grouped them with the genotypes of those markers. The BIL plants with non-parthenocarpic parental homozygous genotypes at the est_cpa03j24 marker locus (nearest to *Cop8.1*) expressed a lower level of parthenocarpy (5.6%), and those with parthenocarpic parental homozygous genotypes at the same locus showed a higher level of parthenocarpy (56.1%; data not shown). This demonstrates the effect of *Cop8.1* in raising the level of parthenocarpy. However, in case of the other QTL, *Cop3.1*, we could not detect positive effect, furthermore, a significant decrease of parthenocarpy level

Fig. 3 Comparison of three linkage maps (*left* ALF2, *middle* LWA2010, *right* NAF2). The *lines* drawn to the side of respective chromosomes represent the range of detected QTL regions with a LOD score higher than 2.5. The *black bars* represent regions with a LOD score higher than the threshold calculated using 1,000 permutation tests (ALF2, 3.7; NAF2, 3.6)
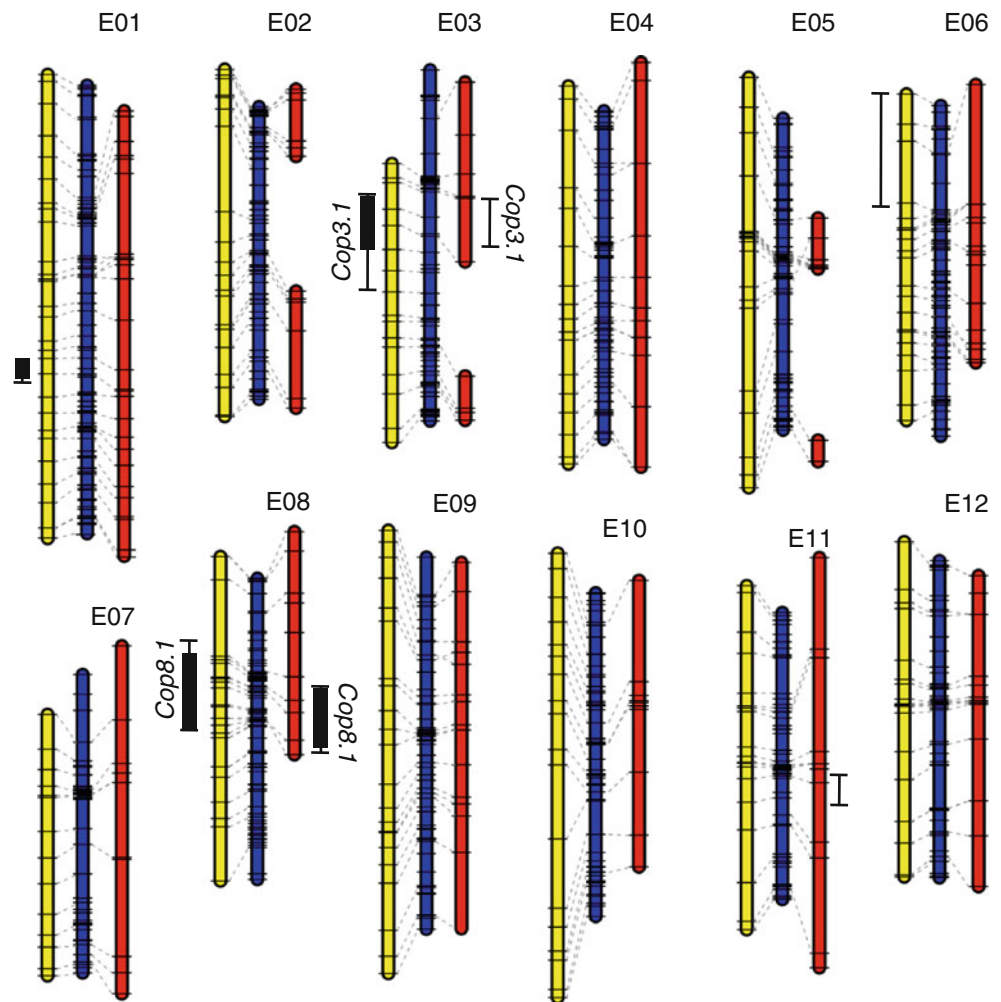
was detected in plants homozygous for the A allele at *Cop8.1* locus (Table 3).

Verification of the effectiveness of the markers nearest to the parthenocarpic QTLs

In the AAF2, KAF2, and SAF2 F$_2$ populations, non-parthenocarpic parents did not develop normal-sized fruits without pollination; therefore, the parthenocarpic characteristic of AE-P03 is clear. Several markers linked to *Cop3.1* and *Cop8.1* were used to screen for polymorphism between the parents. Among them, emf21H22 and emh11J10, which are located at the ends of the LOD peaks of the QTL detected on chromosome 8, were polymorphic [fragment length (Aodaimaru/Kamonasu/Satsuma-Shironaganasu/AE-P03) are emf21H22: 131/131/125/135 bp; emh11J10: 277/277/277/275 bp]. We selected 40 F$_2$ plants with parthenocarpic parental homozygous genotypes and 40 F$_2$ plants with non-parthenocarpic parental homozygous genotypes at the above two markers. We grouped F$_2$ progeny according to the genotypes of the two markers and calculated the mean values of the level of parthenocarpy

(Table 4). The plants with parthenocarpic parental homozygous genotypes showed extremely high levels of parthenocarpy (up to 90%), and the plants with non-parthenocarpic parental homozygous genotypes showed no fruit development (Table 4).

Discussion

To detect parthenocarpic QTLs in eggplant, we conducted genetic analyses using two F$_2$ populations (ALF2, NAF2). The levels of parthenocarpy showed a continuous distribution within the range of the parents, and transgressive segregation was not observed (Fig. 2). In conjunction with results of Shapiro–Wilks test, suggesting that these data did not follow the normal distribution, parthenocarpy was likely controlled by a few genes, but not a single gene.

Linkage maps of ALF2 and NAF2 covered total map distances of 1,414.6 and 1,153.8 cM, respectively. To confirm their genome coverage, we compared them with the integrated linkage map of eggplant (LWA2010, Fukuoka et al. submitted, http://vegmarks.nivot.affrc.go.jp)
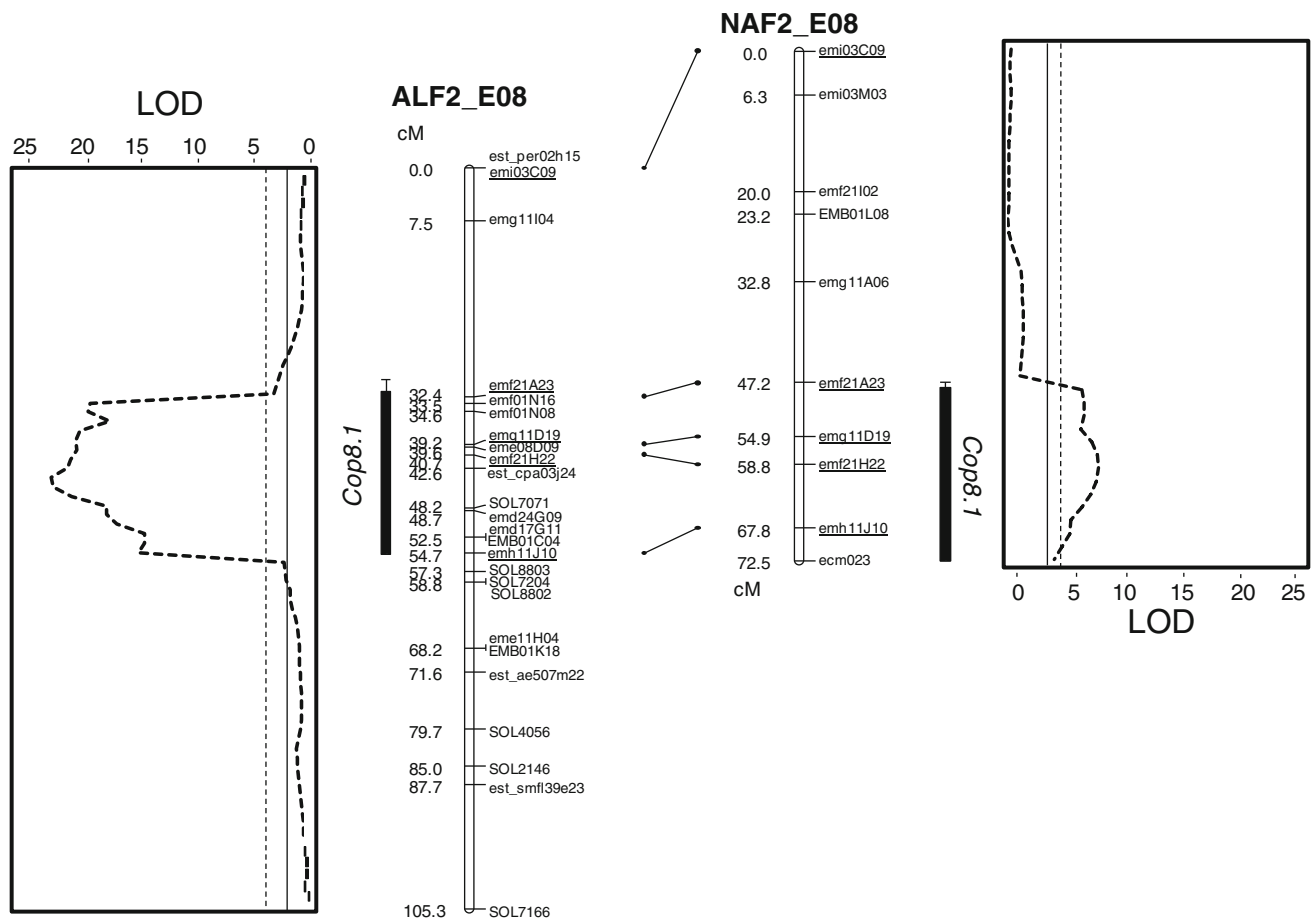
Fig. 4 Comparison of the two linkage maps near the *Cop8.1* locus (*left* ALF2, *right* NAF2). *Underlined names* indicate the common markers; their positions are connected with lines. The *lines* and *black* *bars* represent that the QTL regions were drawn following the criteria described in Fig. 3

using common markers (Fig. 3). The integrated linkage map fully corresponded to the original tomato map, which was estimated to cover the whole genome, using the orthologous SNP markers (data not shown). The ALF2 linkage map covered almost the whole genome, but the NAF2 linkage map covered less than 80% of that of LWA2010 and contained three gaps. The main reason for the relatively low coverage of the NAF2 map is that the maternal parent of NAF2, Nakate-Shinkuro, was one of the breeding origins of AE-P03 (Kikuchi et al. 2008). Consequently, in the genomic region of AE-P03 derived from Nakate-Shinkuro, polymorphisms could not be detected in NAF2. Nonetheless, the ALF2 linkage map sufficiently covered the genome, so no effective QTLs were missed.

QTL analyses performed in this study detected two QTLs (*Cop3.1* and *Cop8.1*) on chromosomes 3 and 8, which both correspond to the tomato chromosome with the same number (Fig. 3; Table 1). The QTL positions detected in this study did not correspond with those of tomato already reported (Fos et al. 2000, 2001; Gorguet et al.

2008; Mazzucato et al. 1998), and there have been no reports of genetic studies of parthenocarpy in other solanaceous fruit vegetables. Therefore, *Cop8.1* and *Cop3.1* are novel parthenocarpic loci in solanaceous species. Results indicate that *Cop8.1* has a major effect, that is, it contributes to the stable expression of the parthenocarpic trait, while *Cop3.1* has a minor and limited effect, depending on the effect of *Cop8.1* (Table 2). Only when the genotype of the marker locus nearest to *Cop8.1* was not homozygous with that of AE-P03 did *Cop3.1* exhibit an effect, but this was not statistically significant (Table 2). The QTL analyses reported here showed that the LOD values of the QTLs detected in the NAF2 population are generally low compared with those in ALF2 (Table 1). One reason may be that the maternal parent of NAF2, Nakate-Shinkuro, has a weak parthenocarpic factor that sometimes triggers a large, malformed fruit set (Kikuchi et al. 2008). This makes it difficult to evaluate the normal, parthenocarpic fruit set.

Cop8.1 was also detected in the population of BILs derived from the same combination of parents (LS1934 and
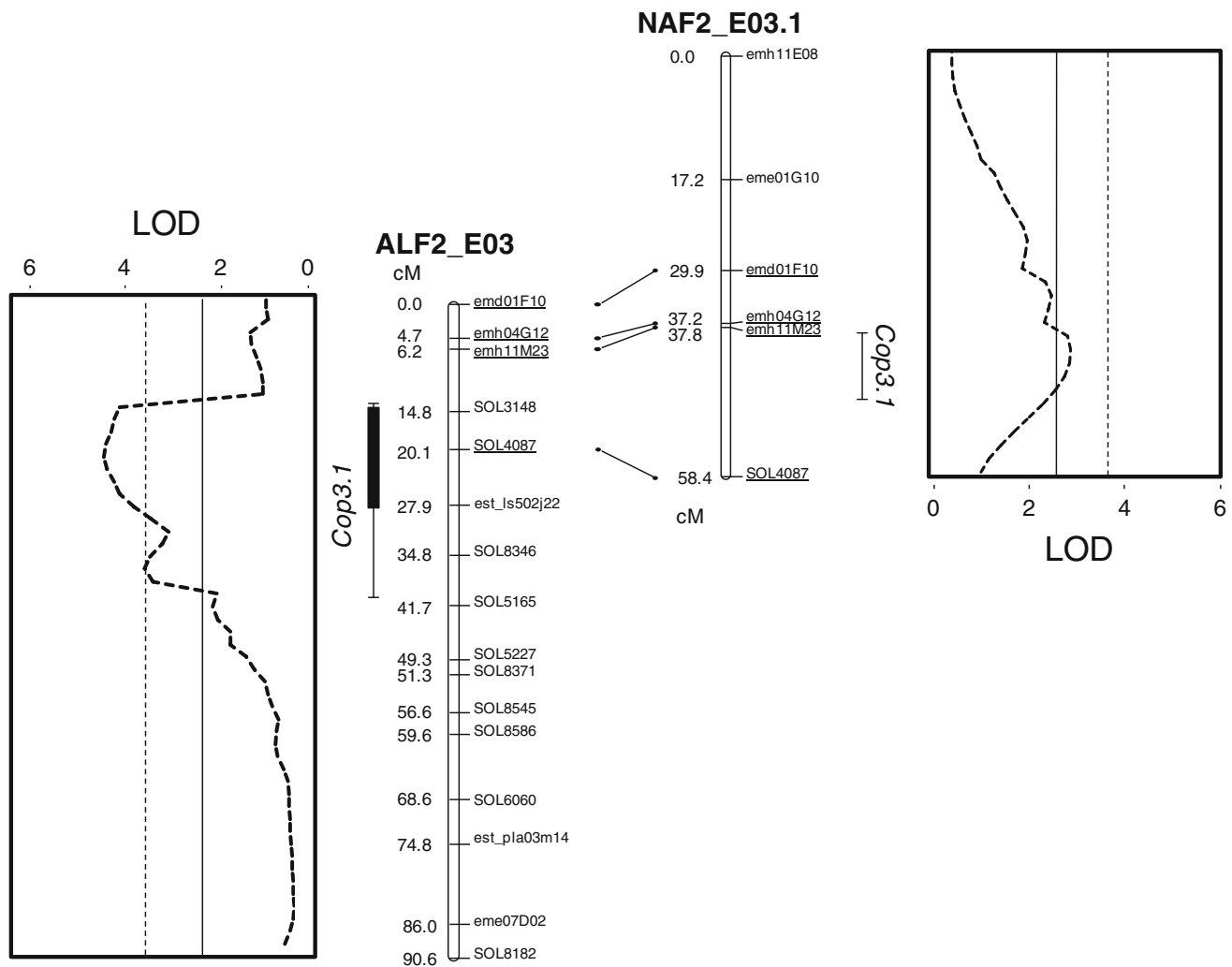
Fig. 5 Comparison of the two linkage maps near the *Cop3.1* locus (*left* ALF2, *right* NAF2). Marker names and QTL regions were drawn following the criteria described in Figs. 3 and 4

Table 1 QTLs detected using composite interval mapping

Population	Chromosome	Flanking markers[a]	Position[b]	LOD	Additive effect[c]	Dominant effect[d]	R^{2e}
ALF2	Chr3	SOL4087-est_ls502j22	23.4	4.2	−11.2	10.9	6.3
	Chr8	est_cpa03j24-SOL7071	42.9	23.8	−30.7	11.9	45.7
NAF2	Chr3	emh11M23-SOL4087	40.2	3.0	−12.1	−15.6	10.6
	Chr8	emh11J10-emf21H22	59.0	7.9	−25.5	−1.6	29.7

[a] Nearest marker is underlined

[b] Expressed in Kosambi cM

[c, d] Positive values indicate that higher-value alleles come from LS1934 and negative values indicate that higher-value alleles come from AE-P03

[e] Proportion of the phenotypic variance explained by the QTL

AE-P03) (Table 3). However, there was no evidence of *Cop3.1* contributing to parthenocarpy (Table 3). The results of the QTL analysis with ALF2 show that it was unlikely that *Cop3.1* had a transgressive dominant effect. It is likely that the environmental conditions affected the expression of parthenocarpy in AE-P03 (Kikuchi et al.

2008). Further studies are necessary to accurately validate the effect of *Cop3.1* under various temperature conditions. In the additional populations of F_2 progeny of crosses between various Japanese non-parthenocarpic cultivars and AE-P03 (AAF2, KAF2, and SAF2), *Cop8.1* contributed to parthenocarpic fruit development regardless of the

Table 2 Mean levels of parthenocarpy (%) of F2 progenies and genotypes around the two identified QTLs

2004–2005[a]

Cop8.1 (est_cpa03j24)	Cop3.1 (est_ls502j22)	Mean	SE	n	
L[b]	L	3.7	2.6	12	a
	H	24.5	4.4	15	acdf
	A	28.0	4.8	10	acd
H	L	39.0	4.8	17	c
	H	62.3	5.7	29	bce
	A	75.9	5.2	12	be
A	L	74.5	5.4	7	b
	H	77.8	4.2	15	be
	A	81.0	4.0	15	be

2007–2008

Cop8.1 (emf21H22)	Cop3.1 (emh11M23)	Mean	SE	n	
N	N	16.7	4.1	5	a
	H	22.4	5.2	13	ad
	A	36.7	5.7	3	ab
H	N	42.9	5.5	17	ab
	H	40.2	5.5	21	abcd
	A	68.2	4.8	9	bc
A	N	77.8	4.8	6	b
	H	69.3	4.4	10	bc
	A	75.0	5.0	6	bc

Values followed by the same letter are not significantly different at $P = 0.05$ by the Tukey–Kramer test. Individuals with missing genotype data at each marker were excluded

SE standard error, *n* number of individuals for which we could determine the genotypes of the nearest markers to the QTLs

[a] Date of the parthenocarpy test: upper, 2004 (Sep)–2005 (Apr); lower, 2007 (Sep)–2008 (Apr)

[b] Genotype of the nearest marker: L, homozygous for LS1934 allele; A, homozygous for AE-P03 allele; N, homozygous for Nakate-Shinkuro allele; H, heterozygous

Table 3 Mean level of parthenocarpy (%) of BILs and genotypes around the two identified QTLs

2010–2011[a]

Cop8.1 (est_cpa03j24)	Cop3.1 (est_ls502j22)	Mean	SE	n	
L[b]	L	6.5	4.0	75	a
	A	2.9	3.1	25	a
A	L	62.5	6.0	22	b
	A	32.3	4.5	6	a

Values followed by the same letter are not significantly different at $P = 0.05$ by the Tukey–Kramer test

SE standard error, *n* number of individuals for which we could determine the genotypes of the nearest markers to the QTLs

[a] Date of the parthenocarpy test: 2010 (Sep)–2011 (Apr)

[b] Genotype of the nearest marker: L, homozygous for LS1934 allele; A, homozygous for AE-P03 allele

Table 4 Mean levels of parthenocarpy (%) of F2 progeny and genotypes around the Cop8.1 locus

2009–2010[a]	Selective markers		Mean	SE	n	
Population	emf21H22	emh11J10				
AAF2	A[b]	A	91.8	0.4	8	a
	AD	AD	0	0	9	b
KAF2	A	A	92.3	0.4	13	a
	K	K	0	0	16	b
SAF2	A	A	98.3	0.3	19	a
	S	S	0	0	15	b

Values followed by the same letter are significantly different at $P = 0.05$ by the Tukey–Kramer test

SE standard error, *N* number of individuals for which we could determine the genotypes of the selective markers

[a] Date of the parthenocarpy test: 2009 (Sep)–2010 (Jan)

[b] Genotype of the selective marker: A, homozygous for AE-P03 allele; AD, homozygous for Aodaimaru allele; K, homozygous for Kamonasu allele; S, homozygous for Satsuma-Shironaganasu allele

genotype of *Cop3.1*. This indicates that by using these two markers, emf21H22 and emh11J10, both linked to *Cop8.1*, we may be able to select fully parthenocarpic individuals at the seedling stage from a wide range of genetic backgrounds (ESM_2). Individuals of the three populations homozygous for the genotype of AE-P03 at the locus of the nearest markers to *Cop8.1* showed extremely high levels of parthenocarpy compared with ALF2 and NAF2 (Tables 2, 4). The main reason was that only three flowers were used to validate parthenocarpy, so plants were under the light burden of fruit-setting. In contrast, the individuals of the three populations homozygous for the genotypes of the non-parthenocarpic parents at the same marker locus showed no parthenocarpic fruit-setting, unlike the ALF2

and NAF2 populations (Tables 2, 4). These results support the conclusion by Kikuchi et al. (2008) that environment influences parthenocarpy.

Yoshida et al. (1998) suggested that the parthenocarpy of Talina (paternal parthenocarpic parent of AE-P03) was controlled by a single dominant gene from segregation tests conducted with the progeny derived from a Talina × EPL1 (Japanese non-parthenocarpic cultivar) cross. Our *Cop3.1* and *Cop8.1* are likely to have been derived from Talina. The genome-wide markers of eggplant reported by Nunome et al. (2009) and Fukuoka et al. (submitted, http://vegmarks.nivot.affrc.go.jp) enabled us to perform QTL analyses with an original scoring method for parthenocarpy as a quantitative characteristic.

In greenhouse cultivation of eggplant, unfavorable conditions such as high or low temperature and a closed environment decrease the amount of fertile pollen in the air, thereby restricting successful fertilization. As a practical measure, synthetic auxins such as 4-chlorophenoxyacetic acid are used to improve the fruit-setting rate. However, exogenous application of 4-chlorophenoxyacetic acid takes time and places a heavy burden on workers. As an ultimate solution, Saito et al. (2009) developed a parthenocarpic cultivar, Anominori, for which Talina, a paternal line of AE-P03, was used as the parthenocarpic resource. Anominori does not have an agronomically unfavorable trait like those seen in parthenocarpic tomato resources (Fos et al. 2000, 2001; Gorguet et al. 2008; Mazzucato et al. 1998; Saito et al. 2009). We confirmed that the genomic region of the main QTL, *Cop8.1* was derived from Talina using SSR markers in all parthenocarpic lines developed at NIVTS (data not shown). Thus, the flanking DNA markers suitable for selecting parthenocarpic genes may be useful as a new tool for developing a wide variety of parthenocarpic eggplant cultivars aimed at practical use. As breeding parthenocarpic cultivars in eggplant takes a lot of time and labor (Saito et al. 2009), this finding enables for the first time a systematic breeding program using DNA markers for parthenocarpic cultivars in solanaceous species. Moreover, past studies reported that synteny was conserved in chromosome 8 between eggplant and tomato (Doganlar et al. 2002; Wu et al. 2009). Therefore, the tomato genome could be used as a powerful tool for determining the genomic structure of eggplant. We hope also to reach the advanced goal of isolating *Cop8.1*. Considering the novelty of these QTLs, finding the detailed genetic and physiological mechanism of parthenocarpy would affect not just the study of eggplant, but also the study of all solanaceous fruit vegetables.

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