RosBREED's Community Breeders' Page

Fast-tracked MAB pipelining in peach: the *endoPG* genetic test for fruit texture type By Cameron Peace

A genetic test exists for predicting whether peach fruit will have the texture of freestone melting flesh (FMF), clingstone melting flesh (CMF), clingstone non-melting flesh (CNMF), or clingstone non-softening flesh (CNSF), as described in the "Jewel in the Genome" of August 2010 (Figure 1). The genetic test is based on endoPG genes present at the Freestone-Melting flesh (F-M) locus. RosBREED is translating the promise of this Jewel into specific tools and information for peach breeders to use in their ongoing breeding decisions. At the risk of mixing metaphors... Using Demonstration Breeder John Clark's analogy of "keys" to his "shiny new Lexus" (see August 2011 issue's Breeder profile; www.rosbreed.org/about/project-management/ participants/demonstration-breeders), the endoPG genetic test is a key to start the breeding engine for a

high-tech drive to superior new cultivars.

How can the endoPG key be used?

The endoPG genetic test opens new peach breeding opportunities – in marker-assisted parent selection



Figure 1. Typical fruit softening profiles for genotypes of the *F-M* locus as determined by the *endoPG* genetic test (averages from a study of 21 FMF, 7 CMF, 8 CNMF, 5 CNSF, and 1 stony hard peach and nectarine cultivars, supported by the National Research Initiative of the USDA CSREES (now USDA NIFA-AFRI) grant number 2005-35300-15463).

(MAPS), marker-assisted seedling selection (MASS), expanded genepools, guiding and monitoring more fine-tuned softening characteristics, simultaneous genetic gain in other traits...

Parent selection. As with any predictive DNA-based genetic test that views the DNA encoding the trait(s) of interest, screening a peach plant for its *endoPG* genotype provides a direct description of its genetic potential. Probably the most effective use of *endoPG* is to describe the fruit texture genotype of any prospective breeding parent (Figure 2). While the *F-M* fruit type (phenotype) of most parents will already be known, predicting progeny outcomes is problematic if parents are heterozygous.

GENETIC	AVAILABLE ALLELES		FRUIT TEXTURE EFFECTS		F
TEST	Primary	Secondary	STONE-FLESH ADHESION	I FLESH SOFTENING TYPE	FRUIT TEXTURE
	, F dominant	F ₁₀₉ , F ₂₀₃ , F ₂₀₅ , F ₂₀₇ , F ₂₃₁	freestone	melting	FMF
endoPG	f	f ₂₀₉ , f ₂₁₁ , f ₂₁₃ , f ₂₁₅ , f ₂₂₇ , f ₂₂₉ , f ₂₃₃ , f ₂₃₅ , f ₂₃₇ , f ₂₇₇ , + many from other specie	clingstone	melting	CMF
	f1	f1 ₁₉₉ ,f1 ₂₀₁	clingstone	non-melting	CNMF
	1 f2 recession	f2 _{null}	clingstone	non-softening	CNSF
	CULTIVAR Fu Pri	nctional genotype mary Secondary		250% FMF	F ₂₀₅ f ₂₂₉ ,
	Chinese Cling f	<u>f229</u>	Chinese Cling	25% CMF	1 205 " null 1 205 11 201
of cultivar functional	Clayton FS Contender F	5 F 205 5	f ₂₂₉ f2 _{nul}	25% CNM	F fil ₂₀₁ f2 _{null}
genotypes	Bright Sweet 15 BY01P6245 Ff Carmen F \$ Carmon # \$	1 F205 f1 201 5 F205 \$ 8 f1 8	F205 f120 BY01P6245	Example of in For fresh-ma cull CNMF se a more effici	nformation use: arket breeding, eedlings or make ent cross

Figure 2. Use of the *endoPG* genetic test for peach fruit texture type.

Seedling selection. The *endoPG* genetic test can predict fruit texture type prior to actual fruiting. If a large population contains two or more types and that is one more than wanted, a breeder can efficiently cull the unwanted types prior to field planting of seedlings.

Crossing between cultivated genepools. Developing new melting flesh cultivars for the fresh market could now involve crossing with nonmelting canning peaches to infuse germplasm with alleles for more intense yellow color or introgress specific disease resistances. The non-melting allele could then be purged in the second generation with MASS, purged for the third generation by MAPS for homozygous melting flesh



Figure 3. Freestones that have become slightly clingy, or clingstones that have freed up a bit? **A** is the former ('Earliglo', Ff, FMF but semi-cling) and **B** is the latter ('Bev's Red', ff, CMF but semi-free). Visual classification of fruit type can be confusing for such early-season cultivars, whereas endoPG reveals the underlying genetics.



Figure 4. An "air-free", but otherwise CNMF, fruit – from one of Tom Gradziel's breeding lines. Its f1f1 genotype means that it should not be free at all. But an almond ancestor may have imparted an allele for airfreeness at a separate and unknown locus.



types, or monitored and tolerated in heterozygous form. Similarly, desirable attributes from melting flesh germplasm can be readily incorporated in canning peach breeding. Traditionally, U.S. melting and non-melting germplasm pools have been separated – *endoPG* can be the matchmaker.

Knowing which traits are not controlled by this locus. The test can reveal what the genetic potential of an individual is - such as unambiguously classifying as either freestone or clingstone an early season variety that has fruit with semi-attached flesh (Figure 3). The test can also discern when traits are *not* directly influenced by genetic variation at the locus. Example 1: Consistent quantitative degrees of clinginess/ freeness do not appear to be associated with the locus. Example 2: A breeding line in the canning peach program of demonstration breeder Tom Gradziel is "air-free" (i.e, in warm seasons, the flesh detaches so cleanly from the pit that an air pocket is formed, yet the flesh is non-melting!) (Figure 4). Example 3: The stony hard trait controlled by the as -yet unmarked Hd locus is not endoPG-controlled. In fact, the stony hard trait masks F-M fruit type unless ethylene is applied to ripe fruit. But the endoPG test is useful in revealing this underlying fruit type, and helps directed combination of fruit texture types. Note that CNSF fruit, homozygous for endoPG's f2 allele, have a very similar softening profile to stony hard! (Figure 1)

Quantitative variation in softening. A Mendelian trait locus like *F-M* is a hotspot for useful quantitative genetic variation that can be exploited when you know the controlling gene. Major gene mutations (such as deletions or rearrangements) typically underlie major phenotypic differences. For *endoPG*, we have an SSR marker that can distinguish "secondary" alleles among peach cultivars that appear to be associated with various rates of softening and differences across a fruit. The effects of further alleles from exotic sources such as wild peach species or almond can be investigated because of the *endoPG* test that detects them.

Indirect selection for other traits. Finally, breeders will wonder whether selecting for a certain *endoPG* allele will indirectly select for other traits. Previous studies indicate QTLs for fruit quality traits such as sweetness and acidity are linked to *F-M*. RosBREED is using *endoPG* and nearby markers

as genetic handles for quantifying such effects and determining linkage phase between alleles of each trait locus in prospective parents. Linkage among trait loci gives breeders exploitable opportunities in their quest for efficient packaging of alleles across the genome for excellent fruit quality.

How is RosBREED cutting the keys for each peach demonstration breeder?

For peach breeders to use the *endoPG* genetic test, it is critical that crop-wide *validation* (stage 4 of the MAB Pipeline, see above) and assessment of breeding *utility* (stage 5) are conducted first. The test is being fast-tracked through these stages in 2011, providing preliminary experience of what will be a more detailed system once all RosBREED infrastructure is in place.

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Evaluation of representative germplasm. To comprehensively and efficiently represent breeding germplasm, the peach Crop Reference Set for all U.S. breeders is bolstered with Breeding Pedigree Sets specific for each program of RosBREED's brave peach Demonstration Breeders. Breeders, breeding trainees, and support staff conduct detailed performance evaluation for fruit quality and other valuable traits each season. The second of three seasons is almost complete. Leaf samples collected last year by breeders were converted into purified DNA and screened with the *endoPG* genetic test via PCR and a high-throughput capillary electrophoresis system in RosBREED's Genotyping Center by the meticulous lab crew of Nahla Bassil (USDA-ARS Corvallis, OR). Next, the Clemson peach team of breeder Ksenija Gasic and trainee Terrence Frett translated the resulting peaks into genotypes for each sample. This genotyping revealed the primary and secondary alleles carried by each of the ~1000 included important breeding parents, ancestors, and seedlings. More than 20 secondary alleles were detected (several never before reported!) and the legitimacy of each required inheritance checking. Inclusion of control cultivars with previously known *endoPG* genotypes helped.

Validation. With phenotypic and genotypic data in hand, statistical analyses for validation can be conducted. The Validation stage identifies the breeding lineages in which the genetic test holds. The Pedigree-Based Analysis program FlexQTLTM is used, although this powerful tool is not needed for something as simple as ensuring that the primary alleles of *endoPG* are predictive of *F-M* fruit type or finding lineages in which they are not. Comparing fruit evaluations of the 2010 season with *endoPG* genetic test results, phenotypes were observed to match genotypes for most samples. Most non-matching cases so far can be attributed to mis-scoring of genotypes or phenotypes (which were then double-checked and corrected). Other cases involve introgressions from related species giving some strange phenotypes in certain lineages. Association of *endoPG* marker alleles with other fruit quality traits will involve FlexQTLTM in the next month or two.

Utility. The Utility stage determines, for an individual breeding program, the functional alleles carried by each potential breeding parent. For the peach demonstration breeders, information on important breeding parents in their Breeding Pedigree Sets is confidential. However, the functional genotypes of Crop Reference Set cultivars were developed for public knowledge: available <u>here</u>.

Other trait loci. From later this year, analysis of high-resolution genotypic data from peach genome scans of the reference germplasm will help determine how other loci across the genome interact with the *endoPG* influence and control other fruit quality traits.

How can other stone fruit breeders get their keys?

Each peach breeding program tends to have its own unique germplasm. The ability to self and the quick generation time relative to other tree fruit crops mean that programs soon develop signature genepools. For distinction in the marketplace of prospective growers, this individuality is useful. For taking advantage of genetic tests, it underscores that Utility assessment need to be conducted for each breeding program. Therefore, once Validation has determined for the crop as a whole that a genetic test is generally predictive (like *endoPG*) or only in certain lineages (like for resistance alleles introgressed from exotic sources), breeders must be convinced that a genetic test has utility for their own local breeding material. Then, functional genotypes are obtained for plants of interest.

Utility of genetic tests for major trait loci. For genetic tests for a major locus like *endoPG*, where the functional mutation itself is assayed, validation need not be a big effort. Most breeders probably need little convincing that such markers are predictive and simply seek the functional genotypes of their prospective parents – rather than bothering to compare phenotypic and genotypic data for a large germplasm set. But some programs include exotic germplasm that could introduce new alleles at the locus or at interacting loci. Suspicion of interacting exotic alleles in specific lineages might demand an independent observation of statistically significant association, perhaps in a small segregating population. Similarly, breeders of one stone fruit crop could springboard from discoveries in another crop by a simple test of association between marker alleles and major phenotypes (e.g., apricot breeders may want to do this to see if *endoPG* influences the freestone character in that crop too). For tests where there is some recombination distance between the trait locus and markers used, a breeder will probably prefer to confirm linkage phase for each parent, requiring that phenotypic data is available.

Utility of genetic tests for quantitative trait loci. Most powerfully, and especially useful for quantitative trait loci, a genetic test's utility for an individual breeding program is discerned by Pedigree-Based Analysis. This approach combines 1) publicly archived genotypic, phenotypic, and pedigree data on a Crop Reference Set with 2) further such data collated by each breeder on germplasm representing their own additional important breeding parents and selections. Modules of RosBREED's Breeder's Toolbox, with FlexQTL[™] included, are being developed to streamline the analysis and interpretation of this data – to enable marker-assisted breeding for all Rosaceae breeders. Coming soon for you all!

Details for running the *endoPG* genetic test can be found <u>here</u>.