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Inheritance and linkage relationships of isozyme and morphological loci in cucumber (*Cucumis sativus* L.)

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Abstract Twenty-one polymorphic and 17 monomorphic cucumber (*Cucumis sativus* L.) isozyme loci were identified in 15 enzyme systems. Seven of the polymorphic loci (*Ak-2, Ak-3, Fdp-1, Fdp-2, Mpi-1, Pep-gl*, and *Skdh*) had not been described previously. Segregation in F_2 and BC families for isozyme and morphological loci demonstrated agreement with the expected 1:2:1 and 1:1 segregation ratio ($P<0.01$). Nine morphological markers were found to be linked to isozyme loci and were integrated to form a map containing four linkage groups spanning 584 cM with a mean linkage distance of approximately 19 cM. Linkage groups (A to D) contain the following loci in genetic order: ***A*** *psl, Pep-la, B, Per, dm, Pgm, Mpi-1, Idh, Ar, Fdp-1, Ak-2, Pgd-1, Mpi-2* and *gl*; ***B*** *lh, Mdh-2, Pep-gl, Pgd-2, Fdp-2, Ccu, Mdh-3, Ak-3, ll, de, F* and *Mdh-1*, and *Gr*; ***C*** *cor, Gpi*, and *Skdh*; ***D*** *Tu* and *ss*. This study detected four new linkages between morphological markers (*dm-psl, de-ll, ll-F*, and *de-F*) and confirmed previously reported linkages, *dm-Ar* and *Tu-ss*. The isozyme/morphological map constructed in this study led to a more comprehensive understanding of the genetic relationships between several economically important traits.

Key words Isozymes · Genetic markers · Disease resistance · Genetic map

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Introduction

Unlike visual markers, molecular markers are relatively abundant and are not pleiotropic to genes of economic importance (O'Brien 1990). Isozymes, restriction fragment length polymorphism (RFLP), and randomly amplified polymorphic DNA (RAPD) can assist in the construction of linkage maps to include economically important traits (Landry et al. 1987; Stuber et al. 1992; Kennard et al. 1994).

The paucity of isozyme variation in most organisms has limited its use. Nevertheless, it has been employed successfully for the confirmation of hybridity (Lo Schiavo et al. 1983), detection of somatic variation (Lassner and Orton 1983), description of mating systems (Ritland 1983), estimation of gene dosage effects (Birchler 1983), genome identification (Pierce and Brewbaker 1973), measurement of genetic variation (Brown and Weir 1983), characterization of taxonomic relationships (Crawford 1983), commercial seed purity testing (Arús 1983), and cultivar identification (Nielsen 1985) and protection (Bailey 1983; Staub and Meglic 1993). Allozymes have been used in map construction because of their codominant expression, resilience to environmental modification, lack of pleiotropic and/or epistatic interactions, speed, and low cost (Brown and Weir 1983; Weeden and Wendel 1989).

Linkages have been detected between allozyme coding loci and loci controlling morphological traits (O'Brien 1990). Where such associations are tight (<5 cM), isozyme analysis can be used to increase gain from selection. For instance, the association between an acid phosphatase (*Aps-1*) allozyme and nematode resistance (*Mi*) (Rick and Fobes 1974) or a nuclear male-sterile locus (*ms-10*) and *Prx-2* (Tanksley et al. 1984) in tomato has proven to be effective as a selection tool. Genetic associations between enzyme-coding loci and quantitative trait loci (QTL) such as yield (Frei et al. 1986) and cold tolerance have also been demonstrated (Guse et al. 1988; Vallejos and Tanksley 1983).

Fanourakis and Simon (1987a) placed 13 of 15 economically important morphological traits of commercial cu-

cumber (*C. sativus* var '*sativus*' L.; x=*n*=7; hereafter referred to as *C. sativus*) in three linkage groups. This and other studies (Whelan et al. 1975; Abdul-Haya et al. 1975) has allowed for the placement of 44 of 105 simply inherited morphological traits into six linkage groups (Pierce and Wehner 1990). Many of these associations, however, have been reported without attending recombination values.

Recently, Knerr and Staub (1992) assigned 12 of 14 isozyme loci in cucumber to four linkage groups. Kennard et al. (1994) used RFLP, RAPD, isozyme and morphological markers to identify ten linkage groups in cucumber. Because more linkage groups than chromosome pairs and 40 unlinked loci (1 isozyme, 3 RFLP and 36 RAPD) were observed, more clarification is needed to associate all of the available genetic markers on seven linkage groups. Therefore, a study was designed to: (1) identify additional polymorphic isozyme loci, and (2) determine linkage relationships between isozyme markers and qualitatively inherited morphological traits.

Materials and methods

Germplasm

Seven hundred and fifty-three *C. sativus* and 4 *C. sativus* var *'hardwickii'* R. Alef (a feral or progenitor form of var '*sativus*'; hereafter referred to as *C. hardwickii*) accessions, which represented the entire cucumber germplasm collection available at the time, were obtained from the United States Department of Agriculture, North Central Regional Plant Introduction Station, Ames, Iowa in 1989. These were surveyed for isozyme variation using 51 enzyme systems in this study (Table 1). Accessions possessing putative alleles of previously uncharacterized isozyme loci were used for inheritance and linkage studies (Table 2). Genetic stocks of *C. sativus* possessing an array of monogenic morphological markers were obtained from breeding programs at North Carolina State University, Raleigh, N. C., the U.S. Department of Agriculture, Agriculture Research Service (USDA/ARS), Madison, Wis., and the University of Wisconsin-Madison, Wis. These stocks were screened for isozyme polymorphisms and then selectively mated to test for linkages within and between isozyme and morphological loci. Individuals possessing variation at isozyme loci were mated to genetic stocks homozygous for qualitatively inherited morphological loci and complementary isozyme loci. Selfing and backcrossing of these F_1 families produced segregating populations for linkage analysis.

Sample preparation and electrophoresis

Cotyledons of 15 individuals from each accession were harvested from 7-day-old seedlings germinated and grown in vermiculite in a greenhouse. Samples were bulked for analysis such that approximately 0.01 g of cotyledonary tissue from each seedling was ground in 0.1 mL of a buffer solution containing 0.67 g/l TRIS base and 7.02 g/l TRIS-HCl at pH 7.1. Plant tissue was held at 5°C (<2 h) before horizontal starch gel electrophoresis was performed according to Knerr and Staub (1992). Modified staining solutions of Allendorf et al. (1977), Brewer (1970), and Shaw and Prasad (1970) were used to visualize banding patterns of the 51 enzyme systems examined (Table 1).

Gels consisted of either 42 g or 56 g of a 1:1:1 mixture of hydrolyzed potato starch from Sigma Co. (St. Louis, Mo.), Connaught Laboratories (Willowdale, Ontario, Canada), and Starch Art (Smithsville, Tex.) dissolved in either 300 ml or 400 ml of buffer, respectively. Gel and electrode buffers described by Clayton and Tretiak (1972), Ridgway et al. (1970), and Selander et al. (1971) were used (Table 1). These are referred to in the text as C (pH 6.1 gel and electrode), R (pH 8.5 gel, 8.1 electrode), S-4 (pH 6.7 gel, 6.3 electrode), and S-9 (pH 8.0 gel, 7.8 electrode), A (pH 7.1 gel, 7.0 electrode), and M (pH 8.7 gel, 8.7 electrode). Isozyme variation in malate dehydrogenase (MDH) was visualized by adjusting the S-4 gel buffer to pH 6.2 and the electrode buffer to pH 5.8 (Knerr et al. 1994).

Isozyme loci

To determine the most appropriate buffer system for electrophoresis we took cotyledons from a random sampling of between 10 to 12 accessions of the U.S. collection. Sample extracts and an array of buffers (C, R, S-4, S-9, A and M) were then used to evaluate those enzyme systems that either did not provide adequate resolution or had not been examined in previous studies (Knerr et al. 1989; Knerr and Staub 1992). Subsequently, a random array of 40% of the accessions of the U.S. germplasm collection was evaluated for variation using 51 enzyme systems (Table 1).

On the basis of this initial survey, isozyme banding patterns observed in 15 enzyme systems (AK, FDP, GR, G2DH, GPI, IDH, MDH, MPI, PEP-GL, PEP-LA, PEP-PAP, PER, PGM, PGD and SKDH) were analyzed in 28 F_2 families. Genetic nomenclature follows a form (Knerr and Staub 1992) modified from Richmond (1972). Enzymes are designated by uppercase abbreviations; loci coding for enzymes (uppercase) are designated by the first letter being uppercase and the rest lowercase. If an enzyme is coded by multiple loci, these are designated by numerals and are numbered from the most cathodal to the most anodal. Alleles of a given locus are numbered from most cathodal to most anodal and enclosed in parentheses. The mobility of the most common allele of a locus was designated 100, and all other alleles were assigned a value based on the mobility (mm) of their homomeric protein product relative to that of the allele 100. For example, an allele at locus 1 of FDP which had a mobility 4 mm less than that of the most common allele was assigned the designation *Fdp(1)*-96.

Individuals possessing alternately homozygous alleles for *Ak-2, Ak-3, Fdp-1, Fdp-2, Gr, G2dh, Gpi, Idh, Mdh-1, Mdh-2, Mdh-3, Mpi-1, Mpi-2, Pep-gl, Pep-la, Pep-pap, Per, Pgm, Pgd-1, Pgd-2*, and *Skdh* were mated to produce F_1 individuals. Sources of putative allozyme variation for *Ak-2, Ak-3, Fdp-1, Fdp-2, Mpi-1, Pep-gl*, and *Skdh* are listed in Table 3. Heterozygous F_1 plants were selfed to produce F_2 families and crossed to a recurrent parent (homozygous for one of the alternate alleles at the same locus) to provide BC families. Results of BC progeny analysis were compared to those obtained from F_2 family segregation to determine the inheritance of allozymes.

Morphological loci

Eighty plants in each of 28 F_2 families were used to test for linkage between morphological traits (described below) and isozyme markers. Isozyme variation was recorded in genetic stocks differing in morphological traits (Table 2). Plants possessing unique isozyme phenotypes were then transplanted to the field or greenhouse for evaluation of morphological traits according to Pierce and Wehner (1990) as follows:

(*ll*) Little leaf – Stem small, leaves miniature. Plants were classified by visual evaluation at the mature plant stage and by measuring the leaf area at the fourth node from the terminal whorl using a leaf area meter (LI-3100, LI-COR, Lincoln, Neb.).

(*dvl*) Divided leaf – True leaves partly or fully divided, often resulting in compound leaves with two to five leaflets. Expressivity complete in plants in the three- to four- leaf stage.

(*cp*) Compact – Reduced internode length (2–5 cm), poorly developed tendrils and small flowers.

(*de*) Determinate habit – Vine length shortened (20–60 cm) with stems terminating in flower clusters.

Table 1 Enzymes assayed using specific buffer systems which provided adequate resolution of isozyme loci observed in cucumber (*Cucumis sativus* L.)

Enzyme	Abbreviation	E.C. designation[a]	Buffer system[b]	Number of putative loci observed[c]
Aconitase	AC	4.2.1.3	S-4	1[d]
Acid phosphatase	ACP	3.1.3.2	C	1
Adenylate kinase	AK	2.7.4.3	S-4	5
Alanine aminotransferase	ALAT	2.6.1.2	R	2
Alcohol dehydrogenase	ADH	1.1.1.1	S-9	1
Aldolase	ALD	4.1.2.13	C	1
Alkaline phosphatase	AKP	3.1.3.1	C	1
Aspartate aminotransferase	AAT	2.6.1.1	R	3
Catalase	CAT	1.11.1.6	R	1
Diaphorase	DIA	1.6.4.1	C	3
Fumarase	FUM	4.2.1.2	M	1[e]
Fructose diphosphatase	FDP	3.1.3.11	A	2[d]
Fructokinase	FK	2.7.1.4	S-9	1[d]
Hydroxybutyric dehydrogenase	HBDH	1.1.1.30	R	1[d]
β-galactosidase	β-GAL	3.2.1.23	C	2
Galactosaminidase	GAM	3.2.1.109	S-9	2
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	C	2
Glucosephosphate isomerase	GPI	5.3.1.9	R	2
β-glucosidase	β-GLU	3.2.1.21	C	1
Glutamate dehydrogenase	GDH	1.4.1.2	R	1
Glutathione reductase	GR	1.6.4.2	S-4	5
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	S-4	1
Glycerate dehydrogenase	G2DH	1.1.1.29	R	1
Glycerol-3-phosphate dehydrogenase	G3P	1.1.1.8	R	1[e]
Glucokinase	GK	2.7.1.2	M	1[d]
Hexoseaminidase	HA	3.2.1.52	R	1
Isocitrate dehydrogenase	IDH	1.1.1.42	S-4	1
Lactate dehydrogenase	LDH	1.1.1.27	R	1
Leucine aminopeptidase	LAP	3.4.11.1	C	1
Mannitol dehydrogenase	MADH	1.1.1.67	S-4	1[d]
Malate dehydrogenase	MDH	1.1.1.37	S-4	3
Malic enzyme	ME	1.1.1.40	C	1
α-mannisodase	α-MAN	3.2.1.24	R	1
Manosephosphate isomerase	MPI	5.3.1.8	S-4	2
Methylumbellifferyl phosphatase	MUP	3.1.1.56	S-4	3
Octanol dehydrogenase	ODH	1.1.1.73	S-9	1[d]
Peptidase with glycyl-leucine	PEPGL	3.4.13.11	A	1[e]
Peptidase with leucyl-alanine	PEPLA	3.4.13.11	M	2
Peptidase with leucylglycyl-glycine	PEPLGG	3.4.13.11	R	3
Peptidase with phenylalanyl-proline	PEPPAP	3.4.13.11	S-4	2
Peroxidase	PER	1.11.1.7	A	6
Phosphoglucomutase	PGM	5.4.2.2	R	3
6-phosphogluconate dehydrogenase	PGD	1.1.1.43	S-4	2
3-phosphoglycerate kinase	PGK	2.7.1.10	C	2
Pyruvic kinase	PK	2.7.1.40	R	4[d]
Phosphoglucokinase	PPGK	2.7.1.10	S-9	1[d]
Sorbitol dehydrogenase	SDH	1.1.1.14	S-4	4[d]
Shikimate dehydrogenase	SKDH	1.1.1.25	S-4	1
Superoxide dismutase	SOD	1.15.1.1	R	2
Triosephosphate isomerase	TPI	5.3.1.1	R	2
Xanthine dehydrogenase	XDH	1.1.1.204	M	1[e]

[a] Enzyme commission number
[b] Buffers of Clayton and Tretiak (1972), Ridgway et al. (1970), and Selander et al. (1971) designated as C, R, and S or M, respectively
[c] Loci designated by previous experiment (Knerr and Staub, 1992) or during this survey using standard criteria and nomenclature (Richmond, 1972)
[d] Enzyme systems not previously characterized
[e] Enzyme systems not resolved in studies by Knerr et al. (1989) and Knerr and Staub (1992)

(*gl*) Glabrous – Absence of trichomes on foliage and fruit.
(*cor*) Cordate leaves – Leaves heart-shaped and velvet.
(*Tu*) Tuberculate fruit – Fruit warty with predominant raised areas along their length.
(*P*) Prominent tubercles – Fruit with cone-shaped tubercles (warts) ≥2 mm in diameter at the base.
(*H*) Heavy netting of fruit – Mature fruit having a netted appearance (~2–4 mm squares) usually associated with a thick epidermis and brown coloration.
(*w*) White fruit color – Mature fruit white in color.
(*B*) Spine color – Fruit with black spines 2–3 weeks after pollination.

Table 2 Parental origin and associated morphological markers of genetic stocks used in genetic map construction of cucumber (*Cucumis sativus L*)

Number	Parent[a]	Origin[b]	Allele symbol[c]	Mating partner(s)[d]
1	GY14a	B	*dm, Ar, psl, Ccu*	21, 22, 6, 3, 23, 20
2	WI 2757	A	*dm, Ar, psl, Ccu*	4, 3, 23
3	SMR18	A	*Dm, ar, Psl*	2, 1
4	H19	A	*De, ll, f*	2, 5, 9, 12
5	G421	B	*de, LL, F*	4
6	WI 1379	A	*Bw*	1
7	WI 1983	A	*LL*	15, 18, 13
8	WI 1983	A	*ll*	18
9	WI 1983	A	*de, F, m*	4, 16
10	WI 3418	A	*F*	12
11	WI 5207	A	*ss*	14
12	WI 6415	A	*lh*	19, 4, 10, 17, 18
13	WI 6418	A	*cor*	7
14	WI 6510	A	*B, H*	17, 24, 15, 11
15	WI 6060	A	*w*	18, 7, 27, 24, 16, 14
16	WI 6392	A	*dvl*	15, 9
17	WI 9052	A	*P*	12, 19, 14
18	WI 5610	B	*cp*	15, 7, 8, 12
19	NCG 30	C	*gl*	12, 17
20	8D-4087	E	*f*	1
21	PI 183967	D	*B, ccu, Dm, ar, Psl, f*	1
22	PI 432860	D	*ccu, Psl*	1
23	Straight 8	A	*Dm, ar, Psl*	1, 2
24	Enza 241	F	*F, Ns*	14
25	Sumter	E	*f*	1
26	Wautoma	E	*f*	1
27	G21231	C	*gl*	15
28	8D-2567	E	*F*	25

[a] Indicates *Cucumis sativus* L. except PI 183967=*Cucumis sativus* var '*hardwickii*' (R.) Alef
[b] A=U.S. Department of Agriculture and Agricultural Research Service, Madison, Wis.; B=University of Wisconsin-Madison, Wis.; C=North Carolina State University, Raleigh, N.C.; D=North Central Plant Introduction Station, Ames, Iowa; E=Ferry-Morse Seed Co., Modesto, Calif.; F=Enza Zaden, Enkhuizen, The Netherlands
[c] Gene symbols of morphological markers used for specific comparisons given as: *Ar* (anthracnose), *B* (black spine color), *Bw* (bacterial wilt), *lh* (long hypocotyl), *Ccu* (scab), *cp* (compact), *de* (determinate habit), *dvl* (divided leaf), *dm* (downy mildew), *F* (female), *gl* (glabrous), *H* (heavy netting of fruit), *ll* (little leaf), *lh* (long hypocotyl), *ns* (numerous spines), *P* (prominent tubercles), *pm* (powdery mildew), *psl* (angular leaf spot), *cor* (cordate leaves), *ss* (small spines), *Tu* (tuberculate fruit), *vvi* (variegated virescent), *w* (white fruit color)
[d] No symbol in a mating partner indicates that the alternate allele was present

(*ns*) Numerous spines – Fruit have numerous spines (≥10 per cm^2) 2–3 weeks after pollination.

(*ss*) Small spines – Fruit have tiny spines (≤1 mm in length) 2–3 weeks after pollination.

(*lh*) Long hypocotyl – Seedlings have a hypocotyl length two to three times greater than standard phenotypes (2–5 cm).

(*vvi*) Variegated virescent – Cotyledons yellow, variegated early in development (5–7 days) becoming green within 14 days after emergence.

(*F*) Sex expression – Plants gynoecious (predominantly female) based on the percentage of female nodes in the first ten flower-bearing nodes (Fredrick and Staub 1989). A node was considered female if it possessed at least one pistillate flower. Individuals with a percentage of female nodes greater than 50% were considered to be homozygous or heterozygous dominant (*F–*), and individuals with a percentage lower than or equal to 50% were considered to be homozygous recessive (*ff*) (Kennard et al. 1994).

Families evaluated in linkage studies segregated for one to six characteristics depending on the parents used (Table 2).

Disease resistance

Fourteen F_2 families (80 plants/family) derived from six *C. sativus*×*C. sativus* or *C. sativus*×*C. hardwickii* initial matings were used to evaluate associations between isozyme and disease resistance loci (Table 2). Depending on the specific isozyme-disease comparison made, mapping parents were polymorphic for *Ak-3, Fdp-2, Idh, Mdh-3, Mpi-1, Mpi-2, Pgm, Pgd-2, Pep-pap, Pep-gl,* and *Per* (Table 1). Two families from a cross between 'GY14' (*C. sativus*)×'PI 183967' (*C. hardwickii*) and 'GY14'×'PI 432860' (*C. sativus*) were used to evaluate linkages between *Fdp-2, Idh, Mdh-3, Mpi-1, Mpi-2, Pgm, Pep-pap, Pep-gl, Per*, and resistance to scab (*Ccu*) caused by *Cladosporium cucumerinum* Ellis and Arth. Three families derived from 'GY14'×'PI 183967', 'WI 2757' (*C. sativus*)×'SMR18' (*C. sativus*) and 'GY14'×'Straight 8' (*C. sativus*) matings tested linkage between *Ak-3, Fdp-2, Mpi-1, Mpi-2, Pgm, Pgd-2, Pep-pap, Per*, and resistance to downy mildew (*dm*) caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow. Three families ('WI 2757'×'SMR18', 'GY14'×'SMR18' and 'GY14'×'PI 183967') were evaluated for resistance to anthracnose (*Ar*) caused by *Colletotrichum orbiculare* (Berk. & Mont.) Arx race 1 and tested for linkage to *Pgd-2, Fdp-2, Mpi-2, Pep-pap, Ak-3* and *Mpi-1*. Two families derived from 'WI 2757'×'SMR18' matings were evaluated for resistance to powdery mildew (*pm*) caused by *Sphaerotheca fuliginea* and tested for linkage to *Ak-3, Fdp-2, Mpi-2, Pgd-2, Pep-pap* and *dm*. Three families derived from 'WI 2757'×'Straight 8', 'GY14'×'PI 183967' and 'GY14'×'PI 432860' were examined for resistance to angular leaf spot (*psl*) caused by *Pseudomonas syringae* pv. *lachrymans* (Smith and Bryan) Carsner and tested for linkage to *Fdp-2, Idh, Mdh-3, Mpi-1, Mpi-2, Pgm, Pep-pap,*

Table 3 Sources of putative allozymic variation for inheritance and linkage studies in cucumber (*Cucumis sativus* L.)[a]

Locus[b]	PI	Source
Ak-2(1)	339247	Turkey
	271754	The Netherlands
Ak-2(2)	279469	Japan
	279463	Japan
Ak-3(1)	113334	Peoples Republic of China
	183967	India
	285603	Poland
Ak-3(2)	169334	Turkey
	255937	The Netherlands
	285603	Poland
Fdp-1(1)	192940	Peoples Republic of China
	169398	Turkey
Fdp-1(2)	169383	Turkey
	192940	Peoples Republic of China
Fdp-2(1)	137851	Iran
	164952	Turkey
Fdp-2(2)	113334	Peoples Republic of China
	192940	Peoples Republic of China
Mpi-1(1 and 2)[c]	176954	Turkey
	249562	Thailand
Pep-gl(1)	113334	Peoples Republic of China
	212896	India
Pep-gl(2)	137851	Iran
	212896	India
Skdh(1 and 2)[c]	302443	Taiwan
	390952	Russia
	487424	Peoples Republic of China

[a] Sources are only given for the less common allele of a locus used based on previous studies by Knerr et al. (1989) and Knerr and Staub (1992)

[b] Enzyme loci designation where multiple loci of an enzyme are distinguished by hyphenated numerals and alleles are enclosed in parentheses ( ). Allozymes that occurred in highest frequency were given the mobility designation 100. All other alleles produced protein products relative to allozyme 100 as follows: *Ak-2*(1)=98; *Ak-3*(1)=98; *Fdp-1*(1)=96; *Fdp-2*(1)=98; *Mpi-1*(1)=96; *Mpi-2*(2)=103; *Pep-gl*(1)=98; *Skdh* (1)=98

[c] Heterogeneous population containing alternate homozygous and heterozygous genotypes

Pep-gl, Per. A single family derived from 'GY14'×'WI 1379' was evaluated for linkage between *Pgd-2* and resistance to bacterial wilt (*Bw*) caused by *Erwinia tracheiphila* (E. F. Smith) Holland. Pathogen isolates were provided by J. Dodson, Peto Seed Company, Woodland, Calif. (downy mildew and angular leaf spot) and M. Havey, UW-Madison (scab and anthracnose). Seedling screening tests were conducted according to Abul-Hayja (1975) and Abul Hayja et al. (1975).

Greenhouse and field experiments

F_1 families heterozygous for morphological and isozyme loci were selfed to produce F_2 families used in map construction (Table 2).F_2 families segregating for isozyme and morphological markers were evaluated either as field plantings in the summer of 1993 at University of Wisconsin Research Station, Hancock, Wis. on Plainfield loamy sand (Typic Udipsament; sandy mixed, mesic) or in a greenhouse at Arlington, Wis. in the fall of 1993. Field evaluation of 17 F_2 families included 34 crosses segregating for *ss, Tu, lh, cp, w, B, H, cor, dvl, F, P, ll, gl, vvi* and *de* (Table 2). One hundred plants per F_2 family were planted in 1.5-m row centers and spaced 50 cm apart in the row. Four parental genotypes were grown for morphological comparison within each family.

Eleven F_2 families from 24 crosses were evaluated in the greenhouse for *cp, ll, w, ns, gl, de*, and *F* depending on their genotype (Table 2). Eighty plants per F_2 family along with four parental plants were grown under supplemental fluorescent lights, which extended the photoperiod to 15 h.

Inheritance and linkage analysis

Data were obtained for codominant isozyme loci from F_2 and BC families and analyzed by chi-square analysis for conformity to expected 1:2:1 and 1:1 Mendelian single-factor segregation ratios, respectively. Dominant morphological loci were fit to the expected F_2 3:1 segregation ratio. Data from putative loci which showed significant deviation from expected segregation ratios ($P<0.001$) were excluded from linkage analysis.

Linkage and orders of markers were estimated with MAPMAKER version 2.0 for Macintosh computers (Lander et al. 1987). Marker loci were initially associated with two-point comparisons with a threshold LOD of 3.0 and $r=0.30$. Loci were then placed in sequence via three-point analyses. Scoring errors were detected by checking for double crossovers. Final candidate orders were confirmed with multipoint analyses using a LOD of 4.0. Final maps depicting relationships among isozyme loci and between isozyme and morphological loci were composed using information from 39 individual linkage groups (maps) with common markers as anchor points.

Results and discussion

Isozyme variation

Experiments were conducted with enzyme systems that had either been inadequately resolved or not examined in previous studies (Knerr and Staub 1992). The intent was to determine the inheritance of the observed variation and, after appropriate matings, to characterize the linkage relationships between these and previously characterized enzyme loci. Twenty-one polymorphic and 17 monomorphic isozyme banding regions were identified in 15 enzyme systems. Fourteen loci (*Gr, G2dh, Gpi, Idh, Mdh-1, Mdh-2, Mdh-3, Mpi-2, Pep-la, Pep-pap, Per, Pgm, Pgd-1*, and *Pgd-2*) had been described previously (Knerr and Staub 1992). Seven additional loci (*Ak-2, Ak-3, Fdp-1, Fdp-2, Mpi-1, Pep-gl*, and *Skdh*) were observed using 5 of the enzyme systems of this study (Table 3, Meglic 1994). Variation at AK is coded for by 2 loci each with two alleles [*Ak-2* (100, 98); *Ak-3* (100, 98)]. Likewise, variation at FDP is coded for by 2 loci each with two alleles [*Fdp-1* (100, 98), *Fdp-2* (100, 98)]. The *Mpi-1* locus possesses 2 alleles (100, 96). Polymorphisms in PEP-GL and SKDH are each coded by 1 locus with two alleles [*Pep-gl* (100, 98); *Skdh* (100, 98)].

Single-factor segregation analysis

Segregation at the seven previously undescribed isozyme loci in the F_2 and BC families demonstrated agreement with the expected 1:2:1 and 1:1 segregation ratio ($P<0.01$) (Ta-

Table 4 F_2 single-factor segregation for seven enzyme-coding loci in cucumber (*Cucumis sativus* L.)

Enzyme locus	Number of Families	Observed			Expected			Chi-square	*P*-value
		AA	AB	BB	AA	AB	BB		
Ak-2	Pooled 4	84	155	78	79	160	79	0.400	0.826
Heterogeneity								2.983	0.400
Ak-3	Pooled 4	68	169	83	80	160	80	2.419	0.298
Heterogeneity								11.106	0.015
Fdp-1	Pooled 3	56	129	52	59	119	59	1.996	0.369
Heterogeneity								2.975	0.250
Fdp-2	Pooled 10	176	390	231	199	399	199	7.954	0.019
Heterogeneity								14.120	0.100
Mpi-1	Pooled 3	52	105	40	49	99	49	2.320	0.314
Heterogeneity								7.672	0.300
Pep-gl	Pooled 3	66	119	55	60	120	60	1.025	0.599
Heterogeneity								0.050	0.950
Skdh	Pooled 3	36	76	48	40	80	40	2.200	0.333
Heterogeneity								5.642	0.060

Table 5 Backcross single-factor segregation for seven enzyme-coding loci in cucumber (*Cucumis sativus* L.)

Enzyme locus	Number of Families	Observed		Expected		Chi-square	*P*-value
		AB	AA	AB	AA		
Ak-2	Pooled 2	34	42	38	38	0.80	0.359
Heterogeneity						0.20	0.150
Ak-3	1	23	17	20	20	0.90	0.343
Fdp-1	Pooled 3	58	62	60	60	0.20	0.715
Heterogeneity						2.80	0.250
Fdp-2	Pooled 6	120	116	118	118	0.07	0.795
Heterogeneity						3.93	0.550
Mpi-1	1	26	14	20	20	3.60	0.058
Pep-gl	Pooled 2	44	36	40	40	0.80	0.371
Heterogeneity						1.80	0.220
Skdh	Pooled 2	27	49	38	38	6.40	0.012
Heterogeneity						0.00	1.000

bles 4 and 5). Single-trait goodness-of-fit tests also indicated that the 14 previously described loci (Knerr and Staub 1992) used in this study were heritable and that variation at *Skdh,* which was unresolved in studies by Knerr and Staub (1992), adequately fit predicted ratios. Segregation of previously described morphological (17) and disease resistance (6) traits (Pierce and Wehner 1990) adequately fit predicted ratios ($P<0.01$) in all families evaluated (data not presented; Meglic 1994).

Linkage analysis

Initially, a LOD of 3.0 and $r=0.3$ were chosen as thresholds for the identification of isozyme linkages in cucumber. This LOD corresponds to a 95% confidence level against the false detection of spurious linkages (Lander and Botstein 1986). Of 107 pairwise comparisons, 99, or 91.5% of the linkages between isozyme loci, were placed on a map using an increased LOD stringency of 4.0 (LOD of 4.0=99.5% confidence). In order to place 7 out of the 107 linked pairs of loci with higher recombination frequencies (>0.30) and LODs<3.0, the threshold was lowered to a LOD of 1 and $r=0.4$.

A 270-cM map containing only isozyme loci was constructed (data not presented). It possesses an average recombination value of 0.16 between loci. The greatest recombination fraction between any 2 isozyme loci was 37% (*Fdp-2 – Mdh-2*), and the smallest was 1% (*Mpi-1 – Idh*). Three linkage groups (A-C) were identified. Linkage group A contains 9 loci and group B includes 8 loci. One linkage group (C) possesses 2 linked loci. Two loci (*Pep-pap* and *G2dh*) segregated independently from the other isozyme loci examined.

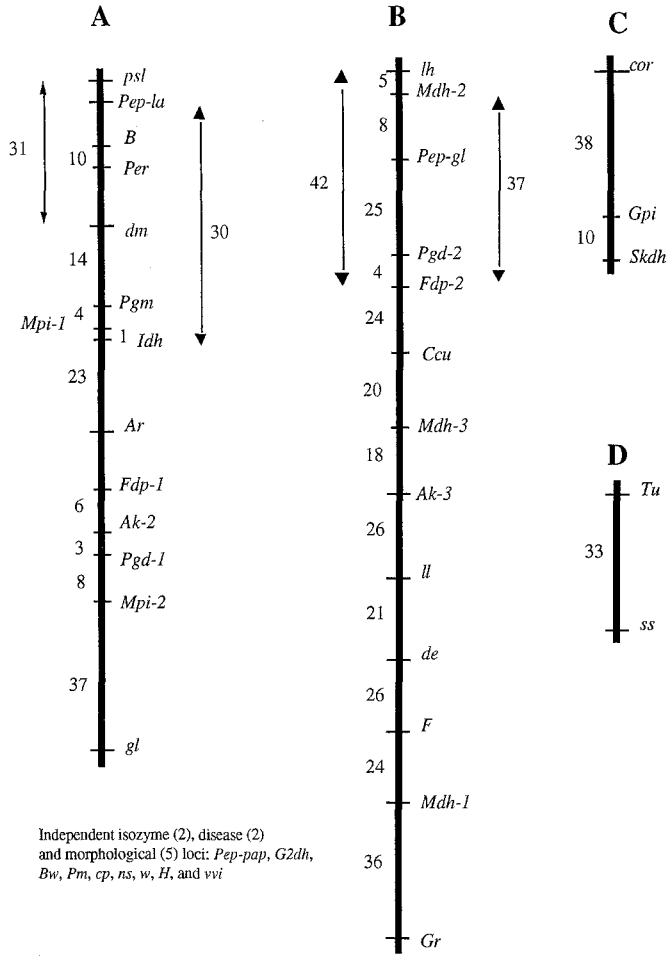

Fig. 1 A linkage map in cucumber (*Cucumis sativus* L.) constructed from the segregation of isozymes and morphological traits in 28 F_2 families

Knerr and Staub (1992) placed 12 of 14 isozyme loci in four linkage groups. They reported two groups comprised of 4 loci each and two with 2 loci each. The average recombination fraction between loci was 23%, and the map spanned 182 cMs in length. Thus, with the 7 additional loci examined in this study, genome coverage exceeded that of the previously reported cucumber isozyme linkage map. The only linkage associations conserved between the two studies were between *Pgm – Idh,* and *Idh – Pgd-1*. These 3 loci retained the same relative order and distances between one another, suggesting that they form a coherent linkage block. In contrast to Knerr and Staub (1992), the *Gr* locus was not found to be associated with *Pgm-1*. The previously reported association between *Pep-la* and *Per* (-Knerr and Staub 1992) helped in positioning *Pep-la* at the end of linkage group A (Fig. 1). The most conserved linkage block observed in this study was *Fdp-2 – Pgd-2 – Ak-3* (linkage group B); it was present in 22 of 39 F_2 families examined.

When all marker types were used in map construction, 9 morphological and 4 disease resistance loci were found to be linked to isozyme loci in four linkage groups (Fig. 1). These associations were integrated into the existing isozyme map to form four linkage groups. This map is 584 cM in length and consists of loci with an average recombination value of 0.20. Three disease resistance loci, *psl* , *dm,* and *Ar* , and 2 morphological loci, *B* and *gl* were placed on linkage group A. Linkage group B was expanded to include 1 disease resistance locus, (*Ccu*) and 4 morphological loci, (*lh*, *ll*, *de* and *F*). One linkage group (group D) is based on the association between two morphological loci, *Tu* and *ss*. The cordate leaf trait (*cor*) was placed on linkage group C but its association with *Gpi* is loose (r=0.38 with a LOD score of 0.95). Two disease resistance loci, (*Bw* and *Pm*), and 5 morphological loci (*cp*, *ns*, *w*, *H*, and *vvi*) segregated independently in 28 mapping populations.

Previously reported linkages between *dm* and *Ar* (Fanourakis and Simon 1987), *Tu* and *ss* (Pierce and Wehner 1990), *Per* and *B*, and *Fdp-2 – Ccu – Mdh-3*(Kennard et al. 1994) were confirmed in this study. The association between *de* and *F* given by Pierce and Wehner (1990) was quantified as having a recombination frequency of 26%. In the present study four previously unreported linkages were detected and placed on linkage group A (*dm – psl*) and B (*de – ll* and *ll – F*) (Fig. 1).

The genome size of a species can be estimated knowing the crossover frequency, the amount of DNA per nucleus, and chromosome number (Beckman and Soller 1983). Given a crossover frequency of 2.1 chiasmata per chromosome arm (Ramachandran and Seshadri 1986) the cucumber genome can be estimated to be at least 740 cM in length. Kennard et al. (1994) used 31 RFLP, 20 RAPD, 5 isozyme and 2 morphological loci to construct a map in *C. sativus* consisting of ten linkage groups spanning 766 cM. Neither this map nor the one constructed in the present study resolved the seven linkage groups fundamental to *C. sativus*.

A disparity exists between the number of linkage groups identified in this study (four) and the number (ten) found by Kennard et al. (1994). This may be due to the number of F_2 families evaluated (28 vs 2, respectively), loci mapped (32 vs 58, respectively) and types of markers examined (isoyme and morphological vs RAPD, RFLP, isozyme and morphological, respectively). The linkages identified in this study were consistent among the families evaluated (homogeneity chi-square values; Tables 4 and 5), and the joining of the linkage groups to from a consensus map was based on common anchor points. A resolution of this apparent controversy might come as attempts are made to join this map with the map of Kennard et al. (1994). This will require identifying anchor points common to both maps. It is possible that some of the more loosely linked loci (e.g., *Idh – Pep-la*, *Fdp-2 – Mdh-2*) will not be retained during map joining. In this case, more linkage groups (>4) may result and, thus, the relationship of the present linkage groups may change.

The map presented here places 21 isozyme loci, 9 morphological and 4 disease resistance loci with a mean map distance of approximately 19 cM. The construction of a more detailed genetic map will require the characterization of more marker loci. Utilization of techniques that de-

tect greater variation than that found with isozymes and/or RFLPs such as amplified fragment length polymorphism (ALFP) or arbitrarily primed polymerase chain reaction (AP-PCR) may allow for increased map saturation (Smith et al. 1994).

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