We show that the degree of resource partitioning between *A. gingivinus* and *A. wattsi pogus* is greater than that between *A. bimaculatus* and *A. wattsi schwartzi* for the three axes along which we observe partitioning. The presence of *A. wattsi* results in both decreased growth rate and increased perch height for *A. gingivinus*. In contrast, no effects of *A. wattsi* on *A. bimaculatus* are observed. The resource partitioning data, therefore, correctly predict the relative strengths of *A. wattsi*'s competitive effect on the two larger *Anolis* species.

Because the *A. bimaculatus* population sizes decrease over time, the mean population density of enclosed *A. bimaculatus* is less than the mean density of enclosed *A. gingivinus*. Another interpretation of our results might be that we observe less competition on St. Eustatius only because of the lower mean population size of animals there. Our data, however, do not support this interpretation. We may partition the St. Eustatius growth and resource use data as before into two classes, one for high *A. bimaculatus* population density (60 to 45) and one for low *A. bimaculatus* density (45 to 30). In the high density class, the mean population size of *A. bimaculatus* (52 per enclosure) is not appreciably lower than the mean population size of *A. gingivinus* (55 per enclosure), and the biomass of *A. bimaculatus* per enclosure is actually higher than the biomass of *A. gingivinus*. Nonetheless, there are no effects of the presence of *A. wattsi* on the growth rate or perch height of *A. bimaculatus* at either high or low *A. bimaculatus* density [Wilcoxon tests without tie correction (*7*)].

Although we have concentrated on the effects of the smaller *Anolis* species on individuals of the larger species, we anticipate that we would obtain similar results from a study of the effects of the two larger species on *A. wattsi*. On St. Maarten it appears that *A. gingivinus* excludes *A. wattsi* from lowland locations, whereas on St. Eustatius *A. wattsi* distribution does extend to sea level. In introduction experiments on an offshore cay near St. Maarten we, together with J. Rummel, have shown that *A. gingivinus* reduces the survivorship of *A. wattsi* in sea-level habitat (*2*). We also show that *A. wattsi* adults can survive for more than 2 years and reproduce in the sea-level habitat. These results suggest that the absence of *A. wattsi* from sea-level habitat on St. Maarten is caused by an interaction with *A. gingivinus* and not by any serious unsuitability of the lowland habitat for *A. wattsi*. In contrast, the extension of *A. wattsi*'s range to sea level on St. Eustatius suggests a weaker effect of *A. bimaculatus* on *A. wattsi*.

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7. We use the following procedure to test for statistically significant differences between growth rate distributions. First, we test for differences among the size distributions of animals from which the growth data were taken. All size distributions for each sex and species are statistically indistinguishable (Wilcoxon tests with tie correction). We then test for differences between the growth rate distributions (ignoring body size) with Wilcoxon tests (without tie correction).
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Manipulations of Endosperm Balance Number Overcome Crossing Barriers Between Diploid *Solanum* Species

Abstract. *Abortion of the hybrid endosperm is the basis for the inability to hybridize many angiosperm species. This has made it nearly impossible to incorporate the valuable characteristics from several wild, diploid* Solanum *species into the cultivated potato* Solanum tuberosum. *But some wild species have "endosperm balance numbers" different from those of most* Solanum *diploid species, and these numbers or "effective ploidies" can be manipulated to create new hybrids.*

Endosperm, a tissue peculiar to angiosperms, is formed by the fertilization of the central cell of the embryo sac by a sperm identical to the one which fertilizes the egg. The endosperm is triploid (3x) in crosses between diploids (2x × 2x) since it receives two sets of maternal chromosomes and one set of paternal chromosomes. After fertilization, the endosperm grows rapidly, becoming the nutritive tissue for the embryo. The nature of the endosperm ranges from an ephemeral tissue, as in bean, to a major tissue in the mature seed, as in corn. However, in almost all angiosperms the survival of the embryo is dependent on the normal development of the endosperm (*1*). Therefore, endosperm is important not only as the major food stuff of man and his livestock, but also because its normal development is necessary to support the development of a viable embryo, thus placing a restraint on our ability to genetically improve crops through sexual hybridization. This limitation is especially important in efforts to introduce into cultivated lines valuable characteristics from wild and exotic germplasm.

There are several types of endosperm dysfunction which prevent crossing (*1*). One type is evident in intraspecific, interploidy crosses. For example, crosses between a diploid and its colchicine-induced tetraploid form generally fail because the endosperm aborts. The cause of this type of dysfunction has been a source of experimentation and debate for over 50 years (*2*). Lin (*3*) clarified the problem by demonstrating that in corn a 2:1 ratio of maternal:paternal chromosomes is necessary for normal endosperm development. In a 2x × 4x cross the maternal:paternal chromosome ratio in the endosperm would be 2:2 and in a 4x × 2x cross, 4:1.

The 2:1 ratio rule is applicable to most intraspecific and many interspecific crosses. However, there are many interspecific crosses whose endosperm development does not conform to the 2:1 ratio rule (*2*). For example, 4x ($2n = 4x = 48$) *Solanum acaule* yields aborted seed when crossed with the cultivated potato, 4x *S. tuberosum* Group Tuberosum. Yet, *S. acaule* crosses readily with 2x ($2n = 2x = 24$) haploids extracted from Gp. Tuberosum, producing seeds with normally developed endosperm (*4*). In the 4x *S. acaule* × 4x Gp. Tuberosum cross, the maternal:paternal ploidy ratio in the endosperm is 4:2 (=2:1), yet the endosperm aborts, while it develops normally in the 4x *S. acaule* × 2x Gp. Tuberosum haploid cross where the maternal:paternal ploidy ratio is 4:1.

An "endosperm balance number" (EBN) hypothesis has been proposed (*2*) in order to establish a single unifying concept concerning endosperm function in intraspecific-interploidy and interspecific crosses. In this hypothesis the genome of each species is assigned an "effective ploidy" (or EBN) with respect to endosperm function by crossing to a species used as a standard. It is the EBN's which must be in a 2:1 maternal: paternal ratio (not necessarily the ploidies) for normal endosperm development. The consistency of this hypothesis has been demonstrated (*5*).

A logical extension of the EBN hypothesis is that EBN differences could act as incompatibility barriers between diploid species. We present evidence, from a simple crossing experiment, that such barriers do exist between diploid *Solanum* species and that manipulation of the EBN ratios in the endosperm can overcome these incompatibilities to produce new and potentially useful interspecific hybrids.

Most of the wild and cultivated diploid species in the subsection Potatoe are intercrossable (*6*). However, there are several species and species groups (series) which seem to be strongly isolated from the majority of diploid species. Representatives of some of these isolated species were used to test the applicability of the EBN concept.

The Mexican diploid *S. cardiophyllum* was used as a representative of the Series Pinnatisecta. There has long been an interest in using the disease resistance of this and the related Series Bulbocastana in potato improvement (*7*). However, there has been very little success in crossing *S. cardiophyllum* and related species with cultivated diploids or other "bridge" species that could in turn be crossed with cultivated diploids (*8*).

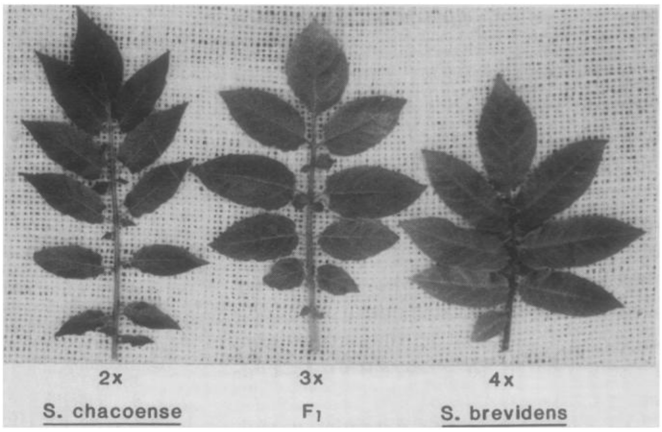

Fig. 1. Leaves from the parents and F_1 of the cross 4x (2EBN) *Solanum brevidens* × 2x (2EBN) *S. chacoense* showing hybridity of F_1.

The closely related diploids, *S. fernandezianum* and *S. brevidens*, are in the Series Etuberosa, which is one of the two series in subsection Potatoe that do not bear tubers (*6*). The species in this series have extreme resistance to frost (*9*) and leaf roll virus (*10*). Interestingly, the only substantiated successful interseries cross involving an Etuberosa species was with a Series Pinnatisecta species (*11*). This suggests that, even though the species in these two series are geographically and evolutionarily widely separated (*6*), the same mechanism may underlie their isolation from other diploids.

Diploid *S. commersonii* is considered to be closely related to and is assigned to the same series, Commersoniana, as *S. chacoense* (*6*), which crosses readily with most diploids. However, there have been no substantiated successful crosses of 2x *S. commersonii* with *S. chacoense* or other diploids.

We performed interspecific crosses using standard techniques in the crossing and identification of hybrids (Table 1). Both *S. chacoense* and *S. verrucosum* had been assigned EBN's of 2 in previous experiments (*5*) and were used as standards in the crosses we report. From the results of crosses 1, 2, 3, and 4 (Table 1) it is clear that *S. cardiophyllum* crossed with 2EBN diploids only when it contributed two genomes as a male, not the usual single one, either in the artificially produced 4x form (cross 3) or through the functioning of 2n (=2x) pollen (cross 4); 2n pollen is pollen which through the functioning of 2n (= 2x) polic ploidy (2n) rather than the normal reduced ploidy (n) of the gamete. There-

Table 1. Interspecific crosses which establish that four diploid *Solanum* species have an endosperm balance number (EBN) of 1. Crosses were made on cut stems in a greenhouse or on intact plants grown in an insect-proof screenhouse. Seeds were extracted from the fruits 5 months after pollination, treated with gibberellic acid (1500 parts per million), and grown in a greenhouse. Six to 15 plants from each cross were grown to maturity, except for cross 11. Hybridity was established by morphological comparisons, and in crosses 3, 4, and 11 triploidy (2n = 3x = 36) was also diagnostic. Chromosome counts were done on root-tip and pollen mother cells. In cross 6, hybridity was confirmed by an irregular meiosis, averaging only 7.9 bivalents per pollen mother cell. All plants were obtained from the Inter-Regional Potato Introduction Station, Sturgeon Bay, Wisconsin.

Experiment number	Cross*		Pollinations (No.)	Fruit (No.)	Average number of seeds per fruit	Condition of endosperm	Ploidy of offspring	Endosperm maternal: paternal ratio	
								Ploidy	EBN
1	2x (2EBN) *S. verrucosum*	× 2x *S. cardiophyllum*	7	6	34	Abortive		2:1	2:1/2
2	4x *S. cardiophyllum*	× 2x *S. cardiophyllum*	5	3	41	Abortive		4:1	2:1/2
3	2x (2EBN) *S. verrucosum*	× 4x *S. cardiophyllum*	17	9	27	Good	3x	2:2	2:1
4	2x (2EBN) *S. chacoense*	× 2x *S. cardiophyllum* (with 2n pollen)	4	4	38	Good	3x	2:2	2:1
5	2x *S. fernandezianum*	× 2x (2EBN) *S. chacoense*	8	8	106	Very abortive		2:1	1:1
6	2x *S. fernandezianum*	× 2x *S. cardiophyllum*	1	1	60	Poor to good	2x	2:1	1:1/2
7	2x *S. commersonii*	× 2x (2EBN) *S. chacoense*	12	9	26	Abortive		2:1	1:1
8	2x *S. commersonii*	× 4x *S. cardiophyllum*	10	10	25	Abortive		2:2	1:1
9	2x *S. commersonii*	× 2x *S. cardiophyllum*	21	14	17	Good	2x	2:1	1:1/2
10	2x *S. brevidens*	× 2x (2EBN) *S. chacoense*	5	4	75	Abortive		2:1	1:1
11	4x *S. brevidens*	× 2x (2EBN) *S. chacoense*	5	3	38	Poor to fair	3x	4:1	2:1

*The plant introduction (PI) numbers of the species used were: *S. brevidens*, PI 218228; *S. cardiophyllum*, PI 255519 or PI 275215 (with 2n pollen); *S. chacoense*, PI 217451; *S. commersonii*, PI 243503; *S. fernandezianum*, PI 320270; and *S. verrucosum*, PI 161173.

fore, *S. cardiophyllum* must be assigned an EBN of 1. This demonstrates that effective ploidy (EBN) differences can function as a barrier to hybridization between diploid species.

Crosses 5 and 6 (Table 1) indicate that the non–tuber-bearing species *S. fernandezianum* can also be assigned an EBN of 1 (*12*). Crosses 7, 8, and 9 demonstrate that *S. commersonii* is also 1EBN. This is especially remarkable because *S. commersonii* is sympatric and considered closely related to 2x (2EBN) *S. chacoense* (*6*).

To demonstrate the potential usefulness of the EBN concept we applied the knowledge of the EBN assignments stated above to create a new hybrid. Diploid (2n = 2x = 24) *S. brevidens* is a non–tuber-bearing species of Series Etuberosa which crosses readily with *S. fernandezianum*; therefore, it should also be 1EBN. In crosses of 2x (1EBN) *S. brevidens* × 2x (2EBN) *S. chacoense*, the hybrid seeds were abortive and the embryos could not be rescued by embryo culture (cross 10, Table 1). However, when colchicine-induced 4x (2EBN) *S. brevidens* was crossed with 2x (2EBN) *S. chacoense* the seeds were much better developed (cross 11, Table 1). Without special treatment, one hybrid seed germinated, producing a vigorous plant that was clearly hybrid (*12*) (Fig. 1). Since the hybrid seeds did not have completely normal endosperm development in the 4x (2EBN) *S. brevidens* × 2x (2EBN) *S. chacoense* cross, either the EBN assigned *S. brevidens* is not precisely 1 or there are other factors involved in endosperm development. Employing a meiotic mutant of 2x (1EBN) *S. commersonii*, which produces 2n pollen and therefore delivers 2EBN to the egg and central cell, we have also produced hybrids between 2x (1EBN) *S. commersonii* and 2x (2EBN) *S. chacoense*.

We have demonstrated that effective ploidy (EBN) barriers exist between diploid *Solanum* species and that, with knowledge of EBN's, ploidies can be manipulated (*13*) to produce new and potentially useful hybrids. Since the EBN concept is probably applicable to many other angiosperm genera (*2*), it could be employed to break crossing barriers between other crops and their wild relatives.

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12. Two of the interspecific crosses were between tuber-bearing and non–tuber-bearing species: 2x *S. fernandezianum* × 2x *S. cardiophyllum* and 4x *S. brevidens* × 2x *S. chacoense*. The F_1's showed hybrid vigor and flowered. Neither type of hybrid could be induced to produce tubers under a variety of temperature and light regimes. However, both types of F_1's produced stolons, which neither *S. fernandezianum* nor *S. brevidens* do.
13. In the experiments described the maternal:paternal EBN ratios in the endosperm were changed by altering the numerical ploidy of one of the parents. However, conceivably the EBN could be changed by altering the dose or character of one chromosome or one gene. We have evidence from experiments in *Datura stramonium* that, at most, two of its 12 chromosomes determine the EBN.
14. This work is a cooperative investigation of the Agricultural Research Service, U.S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

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Changes in the Cell Membranes of the Bullfrog Gastric Mucosa with Acid Secretion

Abstract. *The effective area, resistance, and configuration of the apical and basolateral cell membranes of the bullfrog gastric mucosa were studied as a function of acid secretion rate, by alternating-current impedance methods. The drop in transepithelial resistance with acid secretion is attributed to the great increase in apical membrane area (hence conductance) associated with tubulovesicles. There is no evidence of a change in basolateral membrane resistance or of apical membrane permeability per unit area.*

Acid secretion by the gastric mucosa is one of the more spectacular, complex, and less well understood epithelial transport systems. The large morphological and electrical changes that accompany acid secretion provide clues to its mechanism. With the onset of secretion, there is an enormous increase of apical membrane area due probably (*1*) to the fusion of intracellular vesicles and tubules into the apical membrane (so-called "tubulovesicles") (*2, 3*), and a drop in the transepithelial resistance (R_t) (*4*). Even in the resting mucosa, R_t has the surprisingly low value of a few hundred ohm-cm^2, a value in the range of epithelia with leaky junctions, although in other respects the stomach resembles a tight epithelium (*5*). This low R_t (high transepithelial conductance) is especially surprising in view of the low permeability of the undamaged stomach to H^+ (the so-called mucosal permeability barrier, whose breakdown is associated with peptic ulcers).

Could this high conductance be due to large membrane area? Because of tissue folding and cell surface elaborations such as microvilli, the true membrane area of an epithelium exceeds its nominal flat area, often by a large factor. This multiplication of area would tend to yield a low R_t value normalized to nominal area.

A potentially relevant experimental technique is equivalent electrical circuit analysis by impedance methods, which can yield the apical and basolateral membrane resistances (R_a and R_b) and capacitances (C_a and C_b) of the epithelial cells. Capacitance values are useful as an estimate of the true membrane area, since the capacitances of biological membranes cluster around 1 μF cm^{-2} (*6*). In addition, infolded structures such as lateral intercellular spaces (LIS) and tubulovesicles behave as distributed resistors (*7*) whose electrical properties depend on their ratio of cross-sectional area to length, so that impedance analysis can yield information about ultrastructural geometry nondestructively in an unfixed preparation. We have developed methods for alternating-current (a-c) impedance analysis of a tight epithelium, the rabbit urinary bladder, and found that the analysis yielded values of R_a, R_b, C_a, C_b, and the area/length ratio of the LIS in agreement with independent measures (*8*). By extending this approach to the gastric mucosa, we hoped (i) to monitor (as C_a and C_b) the changes in apical and basolateral membrane areas with HCl secretion, (ii) to establish whether the

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