

RESEARCH PAPER

Genetic diversity in *Egeria densa* and *E. najas* in Jupia Reservoir, Brazil

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Abstract

E.S. Mori, D. Martins, E.D. Velini, C.L. Marino, C.F. Gouvêa, S.M.M. Leite, E. Camacho Palomino, and R.P. Guries. 2012. Genetic diversity in *Egeria densa* and *E. najas* in Jupia Reservoir, Brazil. Cien. Inv. Agr. 39(2):321-330. The aquatic plant species *Egeria densa* (Planch.) Casp and *E. najas* Planchon occur naturally in the Paraná River Basin of southeastern Brazil. Hydroelectric dam construction in the river basin has created several lakes, changing the ecology of the river and altering the population ecology of *Egeria*. Large, dense populations of *Egeria* now grow in Jupia Lake and its tributaries, occasionally blocking hydroelectric turbines. This study is part of a larger project examining environmental changes associated with large dam projects; the research objective of this study was to assess patterns of genetic diversity in *Egeria* populations growing in Jupia and Três Irmãos Lakes and their Paraná River tributaries using genetic markers. Forty-two plants of *E. najas* and 46 of *E. densa* (for a total of 88 samples) were collected from 13 sites. Genotypes were identified by isoenzymes and Random Amplification of Polymorphic DNA RAPD markers. Using a genetic distance dendrogram we grouped all *E. densa* plants into one set and all *E. najas* plants into another set. The plant sample 8a, which presented intermediary morphological characteristics of both species, also presented intermediary genetic characteristics, indicating the possibility that gene introgression between these *Egeria* species may occur. The creation of Três Irmãos Lake appears to have increased some measures of genetic diversity in *Egeria* populations by facilitating outcrossing among previously semi-isolated genotypes. Increased migration of large numbers of seeds and vegetative branches of *E. densa* and *E. najas* into Jupia Lake and its tributaries can account for most of the changes in patterning of genetic diversity observed in these populations of *Egeria*.

Key words: *Egeria*, dam, isoenzyme, genetic distance, RAPD, reservoir.

Introduction

The Paraná River Basin drains an enormous landscape covering parts of Brazil, Argentina, Paraguay and Uruguay before emptying into the

South Atlantic near Buenos Aires, Argentina. Several hydroelectric dams constructed since 1950 have created large reservoirs on the Paraná River, including the 35 sq. km Jupia Lake, thereby altering the natural ecology of this formerly free flowing river. Some aquatic plants, such as the native species *Egeria densa* (Planch.) Casp and *E. najas* Planchon (both Hydrocharitaceae),

previously restricted to riverine margins and pools, have multiplied rapidly in Jupuí Lake and become 'nuisance' plants (Getsinger and Dillon, 1984; Haramoto and Ikusima, 1988; Mori *et al.*, 1999; Oliveira *et al.*, 2005; Martins *et al.*, 2009).

During the rainy season, enormous quantities of *Egeria* branches block turbines at the 1400 Kwatt capacity Jupuí hydroelectric facility, with damage and maintenance costs estimated at approximately 1.5 million dollars (Melloni, 1998). Other Hydrocharitaceae, such as *Lagarosiphon major* (Bowmer *et al.*, 1995; Lambertini *et al.*, 2010), *Hydrilla verticillata* (Bowmer *et al.*, 1995; Les *et al.*, 1997), *Ipomoea aquatica* Forsk (Van and Madeira, 1998), *Elodea canadensis* and *E. densa* (Lambertini *et al.*, 2010) are well-known exotics that have become serious pests in rivers and lakes in the southern United States and western counties in Oregon (Carter and Sytsma, 2001).

Many types of aquatic weeds have caused tremendous damage in lakes, rivers, and irrigation drains throughout the world (Moody *et al.*, 2008). These weeds include the following species: *Egeria* spp. (Martins *et al.*, 2003), *Typha subulata* (Prates *et al.*, 1996), and *Eichhornia azurea* (Ikusima and Gentil, 1993) in Brazil; *Patamogeton illinoensis* (Beziec *et al.*, 1996; Armellina *et al.*, 1996) in Argentina; *E. canadensis* (Bowmer *et al.*, 1984), *Vallisneria Americana* var. *americana* Michaux (Roberts *et al.*, 2001; Robert *et al.*, 1999) in Australia; and *Paspalum distichum* (Shibayama, 1988) in Japan.

E. densa is an aquatic plant that is able to grow in heavy metal contaminated and polluted bodies of water (Malec *et al.*, 2009), multiplying rapidly in polluted lakes.

Ryan *et al.* (1995) and Les *et al.* (1997) found low DNA polymorphism in *H. verticillata* in lakes within the USA. Similarly, Pieterse *et al.* (1984) found low polymorphism using isoenzymes to examine lake populations in Ireland and Poland, as did Ryan (1988) in USA lakes.

In a study on dioecious and monoecious biotypes of *Hydrilla* that occur in the United States, Madeira *et al.* (2004) described an alternative molecular tool using a polymerase chain reaction (PCR) to discriminate monoecious from dioecious plants.

E. densa and *E. najas* species became a problem in Jupuí Lake only when Três Irmãos Lake was constructed on the Tietê River, which enters the Paraná River just upriver from Jupuí Lake, Brazil. To create the Três Irmãos Lake, the Tietê River was diverted for six months, giving *E. densa* and *E. najas* populations an opportunity to cover the then-empty Tietê River channel between Jupuí and Três Irmãos Lakes (Mori *et al.*, 1999).

The dams supply ideal conditions for *E. najas* populations to multiply and invade new areas via asexual reproduction (Mori *et al.*, 1999; Carvalho *et al.*, 2003). They verified vegetative growth of *E. najas* from plant fragments of 5 cm. Therefore, the hydroelectric turbines have contributed to the spread of *E. najas* by breaking up plants during the energy production process.

Both *E. densa* and *E. najas* are submerged, perennial dioecious plants that grow best at water temperatures between 15 and 25 °C. Reproduction is largely asexual via branches that can survive and grow without roots anchored in soil. Reproduction by seed is rare, and it is unusual to find both sexes growing side by side, making pollination by insects difficult (Cook and Urmi-Kbnig, 1984; Kissmann 1991).

No prior information is available on genetic diversity in *E. densa* or *E. najas*, but both are morphologically very similar to *Hydrilla verticillata* (L. F.) Royle, a species that has been the subject of isoenzyme and DNA analyses (Aulback-Smith, 1990). In addition, Verkleij *et al.* (1983) studied 28 populations of *H. verticillata* from Pakistan, India, Malaysia, Indonesia, New Zealand, USA, Panama and Poland, using 18 isoenzymatic systems. Les *et al.* (1997) and Ryan *et al.* (1995) characterized *H. verticillata* using molecular markers.

Power companies and facility managers find it difficult to believe that previously ‘well-behaved’ species such as *Egeria* could change their ecology so dramatically following dam construction, and they question whether other changes are responsible. Examining the current patterns of genetic diversity in Jupuí Lake might suggest whether major genetic change has recently occurred; such knowledge might be useful in predicting future environmental change. The objective of this research was to examine the current genetic diversity in *E. densa* and *E. najas* populations in Jupuí Lake and tributaries of Paraná River using molecular markers. This study is part of a larger project examining the environmental effects of dam construction.

Materials and methods

Plant materials

The map presented in Figure 1 shows the distribution of the thirteen plant sample sites throughout

the Jupuí Reservoir, Tietê River, Pereira Barreto Canal, and São José dos Dourados River, Brazil.

The stems of *E. densa* and *E. najas* are monomorphic, elongate, up to 3 m or more in length, and irregularly branched; branches are borne at “double internodes”, resembling the main stem. Their leaves are opposites or whorled without stipules and are not differentiated into petiole and lamina (Cook and Urmi-Ko“nig, 1984). Reproduction is largely asexual via branches that can survive and grow without roots anchored in soil. Reproduction by seed is rare, and it is unusual to find both sexes growing side by side, making pollination by insects difficult (Kissmann, 1991).

Thirteen sites were selected for sampling. Some sites did not have populations of both *Egeria* species, thus a different number of samples were collected per site. A site along the Sucurí River was originally included in the sampling design but no *Egeria* plants were found in this location.

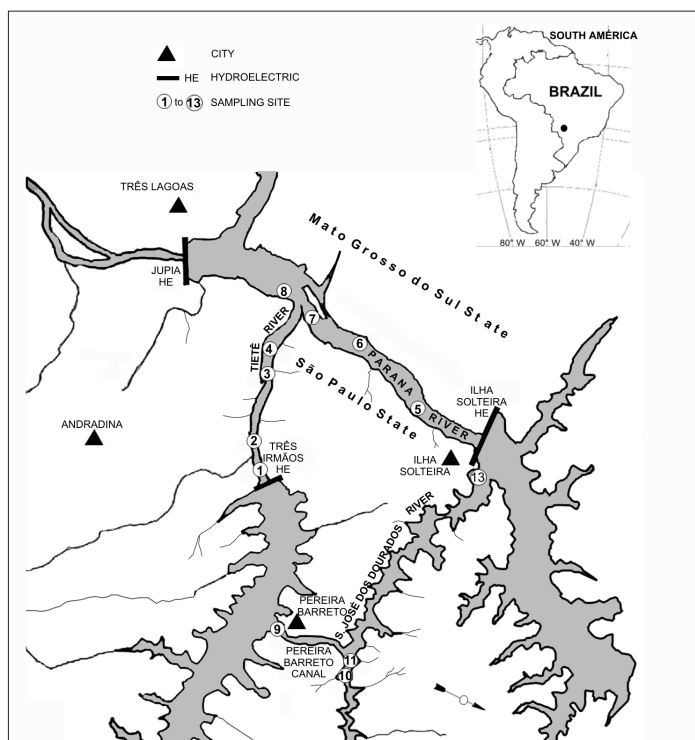


Figure 1. Map showing the sites where *Egeria densa* and *E. najas* are distributed in Jupuí Reservoir, Tietê River, Pereira Barreto Canal, and São José dos Dourados River, Brazil.

Specimen collection

The original sampling design called for collecting plants of both species from seven sub-populations at each of 13 sites (a total of 91 sub-populations for each species) along Jupuí Lake, the Tietê, Sucurí and São José dos Dourados Rivers, and Pereira Barreto Canal, along the border between the States of São Paulo and Mato Grosso do Sul, Brazil (Table 1). In approximately half of the sites, one or the other *Egeria* species was absent from a sub-population (Table 2); a total of 88 plants were collected for the two species (42 *E. najas* plants and 46 *E. densa* plants).

Collections from *Egeria* sub-populations were separated by a distance of at least 200 m. Plant specimens were transferred to an aquarium at the Department of Crop Science, São Paulo State University (UNESP), Botucatu, SP, Brazil. Species identities were confirmed morphologically, and live plants were maintained in the aquarium to provide easy access to specimens for DNA and isoenzyme extractions.

Isoenzyme procedures

Isoenzymes are widely used as molecular markers to understand the genetic relationships within and among populations. Relative to other types of molecular markers (e.g., RAPDs or RFLPs), isoenzymes are relatively inexpensive for population survey work, have a well-established genetic function, and can be conducted with a minimum of sophisticated, expensive electrophoresis equipment (Hedrick, 1971; Rogers, 1972; Nei, 1978; and Wain *et al.*, 1985). The technique used in this study involved minor variations from frequently cited methods. One hundred milligram of fresh leaves from each plant were used to prepare a crude homogenate using the extraction technique of Verkleij (1983). Three gel/electrode buffer systems (Figure 2) were used based on Cheliak and Pitel (1984), Alfenas *et al.* (1991) and Ballve *et al.* (1991), with electrophoresis performed at temperatures near 5 °C. A total of 17 isoenzymatic systems were studied, including AAT, ACP, ADH, CAT, α -EST, β -EST, GDH, G6P, G2D, IDH, LAP, MADH, MDH, ME, PEX, SDH, and

Table 1. Branch collection sites and sample numbers from locations along Jupuí Lake, Tietê River, Pereira Barreto Canal, and São José dos Dourados River, Brazil.

Species	Site	Collection Site	# of Sub-Sample per Collection Site	
<i>Egeria densa</i>	Tietê River	Ed1	1(1) to 1(7)	
		Ed2	2(1) to 2(7)	
		Ed3	3(1) to 3(7)	
		Ed4	4(1) to 4(7)	
	Jupuí Lake	Ed5	5(1) to 5(7)	
		Ed7	7(1) to 7(7)	
<i>Egeria najas</i>	Tietê River	En1a	1(4)	
		En1b	1(7)	
		En2	2(6)	
		En4	4(5)	
	Jupuí Lake	En5	5(1) and from 5(2) to 5(7)	
		En6	6(1) to 6(7)	
		En7	7(1) to 7(4) and 7(7)	
		8a	8(1)	
		En8b	8(2)	
		Pereira Barreto Canal	En9	9(1) to 9(7)
		S. J. Dourados River	En10	10(1)
			En11	11(1) to 11(7)
			En12	12(1) to 12(7)
En13	12(1)			
Total		88 plants		

Note: E.g.: # of Sub-Samples per Collection Site - 13(1) means Sub-Sample 1 of Site En13.

SKDH (Cheliak and Pitel; 1984; Yamada and Guries, 1989).

RAPD procedures

As a comparison to the isoenzyme analysis described above, a sub-sample of plants were also analyzed using Random Amplified Polymorphic DNA (RAPDs) according to the procedure of Williams *et al.* (1990), modified by Grattapaglia and Sederof (1994).

DNA was extracted from 150 mg of leaves ground in 700 μ l of CTAB buffer [100 mM of Tris-HCl (pH 8.0), 1 % hexadecyltrimethylammonium bromide (CTAB), 0.7 M NaCl, 10 mM EDTA, 2% mercaptoethanol and 1% polyvinylpyrimidine (PVP)] and heated to 65 °C for 30 to 60 minutes with shaking. This solution was added to 600 μ L of chloroform-isoamyl alcohol (24:1 v/v); tubes were shaken gently and then centrifuged at 6,000 rpm for 15 minutes. The homogenate was then emulsified again in CTAB buffer, the supernatant was added to 0.5 volume of 5 M NaCl and 2 volumes of isopropanol at -20 °C, and the tubes were shaken gently until DNA precipitated. Following incubation at -20 °C for approximately 12 hours, the solution was centrifuged at 3,000 rpm for 3 minutes. The DNA pellets were washed with ethanol, dried at room temperature and re-dissolved in TE buffer with RNase (1 μ L per 100 μ L of DNA) for 2 hours at 37 °C. The relative amount of DNA for each sample was quantified and stored at -20 °C.

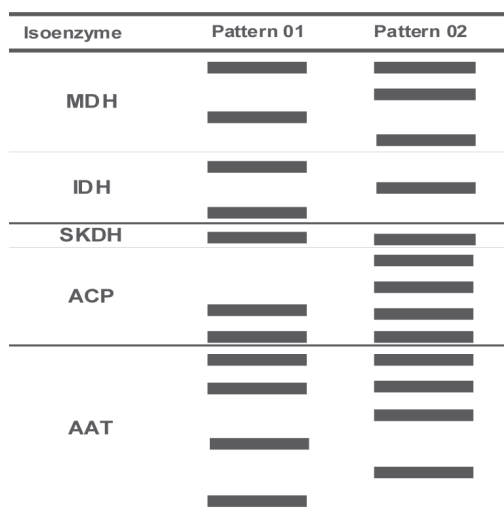


Figure 2. Banding patterns of the five isoenzyme systems of *Egeria densa* and *E. najas*.

Fifty 10-mer primers were tested using an Operon kit; six polymorphic primers were selected for further use in this study. The sequences are as follows: P-01 – GTAGCACTCC, P-16 – CCAAGCTGCC, P-17 – TGACCCGCCT, J-01 – CCCGGCATAA, J-11 – ACTCCTGCGA, and J-17 – ACGCCAGTTC.

Each analysis was performed in a 13 μ l reaction containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton-X, 200 mM for each dNTP, 0.4 mM of each primer, 0.01 mg non-acetylated bovine serum albumin, 5 to 10 mg genomic DNA and 1 unit of Taq polymerase (Promega). The reactions were overlaid with 50 μ L of mineral oil. The amplification reaction included an initial denaturation step of 1 minute at 92 °C, followed by 40 cycles of 1 minute at 35 °C and 2 minutes at 72 °C.

Table 2. The isoenzyme phenotypes identified for each sub-population collection site for *Egeria densa* and *E. najas*.

Collection Site	Species	Phenotype				
		MDH	IDH	SKD	ACP	AAT
Ed1, Ed2, Ed3, Ed4, Ed5, and Ed7	<i>Egeria densa</i>	1	1	1	1	1
8a	?	2	2	1	2	2
En8b		1	1	1	1	1
En1	<i>Egeria najas</i>	1	1	1	2	2
En4 and En7		1	2	1	2	2
En2, En5, En6, En9, En10, En11, and En12		2	2	1	2	2

Genetic analysis

We used the unweighted pair group method with arithmetic averages (UPGMA) algorithm cluster analysis to construct genetic dendrograms using the 75 polymorphic RAPD bands and 19 polymorphic isoenzyme bands (94 bands in total) amplified in each of the 88 individuals.

Result

Only five of the 17 isoenzyme systems studied were polymorphic: MDH, IDH, SKD, ACP, and AAT. Two distinct phenotypes were found for each of these five systems (Figure 2), with five distinct clusters of sub-populations. All *E. densa* plants were included in one group, while *E. najas* was divided into 4 groups (Table 2).

Six of the fifty 10-mer primers tested were polymorphic in one or more sub-populations of both *E. densa* and *E. najas* (Figure 3). Some primers revealed more polymorphisms than others.

A dendrogram constructed using RAPD data cleanly separates the two species without mixing sub-populations or plants. The dendrogram in Figure 3 was built using estimates of Nei's genetic identity between *E. densa* and *E. najas*.

The sample 8a, which showed intermediary morphological characteristics, lay midway between both species in the dendrogram, thus confirming that the sample also has intermediate genetic characteristics.

Discussion

In reservoirs and in large, slow-moving rivers, *Egeria* colonies can multiply to high densities and occupy large areas. Where water flow is rapid, there are typically only a few or no *Egeria* plants growing on the river floor. However, in the channel of the Tietê River, between Jupia and Três Irmãos Lakes, large, dense populations of both species now occur.

The results of this study revealed that all 42 individuals of *E. densa* from six different collection sites throughout the Tietê River and Jupia Lake are identical at isoenzymatic loci. Based upon isoenzyme data alone, we might conclude that a single clone of *E. densa* now occupies the entire study area. *E. najas* populations were slightly more polymorphic and appear to form four groups (Table 2). Similarly, Verkleij *et al.* (1983) reported that all US populations of the exotic plant, *H. verticillata*, sampled in their study had identical isoenzyme phenotypes.

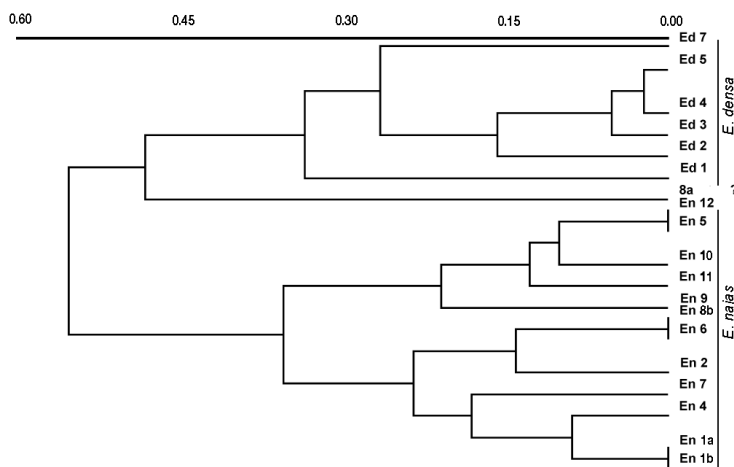


Figure 3. Genetic distance dendrogram of *Egeria najas* and *E. densa* based on Nei's (1978).

The authors concluded that the vast *Hydrilla* populations in US lakes appear to share a single clonal origin most likely arising from a single colonization event.

The limited amount of polymorphism present in the isoenzyme systems studied in *Egeria* spp. cannot provide answers to most questions concerning population differences.

Within each species, almost all sub-populations appear closely related, with genetic distance coefficients higher than 0.25. However, there are a few plants that appear less typical of the species, with coefficients of genetic distance from 0.25 to 0.50. For example, based on morphological characteristics, plant 8a appears to be *E. najas*, but may share some RAPD patterns common to *E. densa*. We have no independent evidence that inter-specific hybridization or introgression is occurring between both *Egeria* species, but the coexistence of these plants in mixed populations, and the modified environment of the rivers and lake, make hybridization a possibility that should be examined more closely.

Most *E. najas* samples collected in the Pereira Barretos Canal and the São José dos Dourados River appeared very similar genetically (genetic distance coefficient >0.25), but only plants collected at site En12 (São José dos Dourados River and Pereira Barretos Canal) and at site En5 (Jupiá Lake) were identical for all RAPD and isoenzyme markers. These results indicate that the canal facilitates the transfer and spread of single genotypes of *E. najas* from the Tietê River via Pereira Barretos Canal into Jupiá Lake or via the Paraná River into Jupiá Lake.

At the En1 site, we collected two ramets of *E. najas* of the same genotype in two different sub-samples at 800 m distance, indicating that large single clonal populations may also exist.

The genetic diversity in the *Egeria* populations observed in Jupiá Lake and its tributary rivers may have originated from different populations in the Paraná River Basin through either sexual or asexual reproduction. The prevalence of sexual reproduction in *E. densa* was modest at best, similar to levels of sexual reproduction observed in *E. najas* (Mori *et al.*, 1999), suggesting that the major source of new diversity in Jupiá Lake is the introduction of vegetative shoots. This pattern of colonization by one or a few well-adapted genotypes is consistent with observations of other aquatic perennials such as *H. verticillata* (Verkleij *et al.*, 1983), *Elodea nuttalli* (Kodono *et al.*, 1997), and *E. najas* (Mori *et al.*, 1999; Martins *et al.*, 2003).

In natural environments, male and female plants of *E. densa* may be separated by hundreds of meters, so pollination is not frequent (Cook and Urmi-Kbnig, 1984). However, the new environmental conditions created in Jupiá Lake following dam construction, e.g., more and larger sub-populations, may actually facilitate outcrossing in *Egeria* and provide more opportunities for interspecific hybridization.

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Resumen

E.S. Mori, D. Martins, E.D. Velini, C.L. Marino, C.F. Gouvêa, S.M.M. Leite, E. Camacho Palomino y R.P. Guries. 2012. Diversidad genética en plantas acuáticas de *Egeria densa* y *E. najas* en el lago de Jupuíá. Cien. Inv. Agr. 39(2):321-330. Las especies acuáticas de *Egeria densa* y *E. najas* ocurren naturalmente en la Cuenca del Río Paraná, sudeste del Brasil. La construcción de una represa hidroeléctrica en la cuenca de este río, creó varios lagos, cambiando la ecología del río y alterando la población ecológica de *Egeria*. Grandes y densas poblaciones de *Egeria* crecen hoy en día en el lago de Jupuíá y sus afluentes, bloqueando de vez en cuando las turbinas de la hidroeléctrica. Como parte de un estudio de cambios ambientales, asociados a grandes proyectos de represas, fueron usados marcadores moleculares para determinar patrones de diversidad genética en las poblaciones de *Egeria* que crecen en los lagos de Jupuíá, Três Irmãos y sus afluentes del Río Paraná. En un total de 13 sitios, fueron colectadas 42 plantas de *E. najas* y 46 de *E. densa*, que suman un total de 88 muestras. Los genotipos fueron identificados por marcadores de isoenzimas y RAPD. A través de dendrogramas de distancia genética se agruparon por separado todas las plantas de *E. densa* y de *E. najas*. La muestra 8a presentó plantas con características morfológicas y genéticas intermediarias de ambas las especies, indicando haber posibilidades de introgresión de genes entre ambas las especies de *Egeria*. La creación del lago de Três Irmãos parece haber aumentado, en alguna medida, la diversidad genética de las poblaciones de *Egeria* por la facilidad de cruzamientos entre genotipos, hasta entonces semi-aislados. La migración creciente de grandes cantidades de semillas y de partes vegetativas de *Egeria densa* y *Egeria najas* dentro del Lago Jupuíá y sus afluentes, pueden explicar los cambios observados en los patrones de diversidad genética en estas poblaciones de *Egeria*.

Palabras clave: *Egeria*, distancia genética, isoenzima, RAPD, represa.

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