Nuclear DNA Content of Basidiospores of Selected Rust Fungi as Estimated from Fluorescence of Propidium Iodide-Stained Nuclei

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ABSTRACT

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The relative nuclear DNA content of basidiospores of five species of *Puccinia,* two of *Uromyces,* and one of *Melampsora* was estimated from the fluorescence intensity of propidium iodide-stained nuclei. Relative to the nuclear fluorescence of *P. graminis,* taken as 1.0, other fungi had the following fluorescence values: *M. lini* (one collection), 2.5; *P. coronata* (two collections), 1.1; *P. hordei* (11 collections), 1.7; *P. recondita* (six collections), 2.0; *P. sorghi* (one collection), 0.8; *U. appendiculatus* (one collection) 6.9; *U. reichertii* (one collection), 1.6; and *U. vignae* (one collection), 4.3. Assuming that the basidiospore nuclei were all haploid and in the same stage of the nuclear cycle, the fluorescence values give an estimate of relative genome size among these fungi. Because of their smaller genome sizes, *P. graminis, P. coronata,* and *P. sorghi* may be more suited for mapping genetic loci than the other fungi measured. In some cases, DNA content within a species differed among collections from different uredinial hosts. Thus, within *P. coronata,* a collection from *Avena sativa* had less fluorescence than a collection from *A. sterilis;* within *P. hordei,* a collection from *Hordeum bulbosum* had more fluorescence than collections from three other species of *Hordeum;* and within *P. recondita,* a collection of *Triticum durum* had less fluorescence than collections from four species of *Aegilops.* This suggests that each rust fungus may have diverged in genome size during evolution on different host species, a possibility in need of further investigation.

Knowledge of nuclear genome size is useful for genetic and physical mapping and can indicate possible evolutionary relationships, especially where ploidy increase may have had a role. For rust fungi, little is known about size, chromosome number, or other aspects of the genome. Accordingly, we have estimated the relative amount of nuclear DNA in several rust fungi selected for availability and, in most cases, for their economic importance in agriculture. Available genetic and physiological information makes several of them candidates for genome

mapping. We were especially interested in learning which of the selected species had the smallest genome size, since small genomes are advantageous for mapping purposes. We also wished to obtain a preliminary indication of variation in genome size among diverse isolates within species.

The relative amount of DNA in nuclei can be estimated from the amount of fluorescence from nuclei that have been stained with DNA-specific fluorochromes. For rust fungi, this has been done in several investigations, principally for comparisons of variants or developmental stages within a given species (12,17, 25–27). However, Wittmann-Meixner (27) described briefly a comparison among several rust fungi. The study was performed with the fluorochrome 4′,6-diamidino-2-phenylindole (DAPI), which binds to AT-rich regions of DNA (15), which, in turn, vary in amount among fungal species (20,28). We have used fluorescence of propidium iodide (PI)-stained nuclei to estimate relative DNA content. PI, now widely used for measuring DNA content by flow cytometry (23), intercalates independently of base pair content (13), as does ethidium bromide (18), and is therefore better suited than DAPI for comparisons among species.

We measured nuclear fluorescence in basidiospores, which generally have two nuclei, each thought to be haploid. For some species, nuclear fluorescence also was measured in urediniospores and pycniospores.

MATERIALS AND METHODS

Telia of nine species of rust fungi were collected from Israel and North America (Table 1). Within species, collections differed with respect to telial host, alternate host, or both (Table 1). All species were long-cycled (macrocyclic). The number of collections within species depended on the availability of telia with germinable teliospores. Collections from Israel were from diverse habitats indicated by region (Table 1).

Most of the telia from Israel were collected after several weeks of summer, after the host had ripened. After collection, leaves with telia were stored in paper bags at 2–4 C for 2 mo to 5 yr. Telia collected in North America were either overwintered outside or given alternate cycles of freezing (−5 C) and thawing (7.5 C) four times per day with 12-h light per day to enhance germinability. Urediniospores of *Puccinia coronata* and *P. graminis* f. sp. *secalis* were used immediately after harvest or after storage at 4 C for up to 1 mo, or 1 yr in the case of *Uromyces appendiculatus*. Pycniospores of *P. coronata* and *P. g. secalis* were used immediately after harvest.

TABLE 1. Species, host, isolate or race, and geographical origin of rust fungus collections, and the spore types used to measure relative DNA content

Collection number	Species	Telial host	Alternate host	Spore types[a]	Isolate or race	Geographical origin[b]
1	*Melampsora lini* (Ehrenb.) Desmaz.	*Linum usitatissimum* L.	Autoecious	B	Race 1	North Dakota
2	*Puccinia coronata* Corda	*Avena sativa* L.	*Rhamnus cathartica* L.	P,B	91-CRBN	Minnesota
3	*P. coronata*	*A. sativa*	*R. cathartica*	U	89-MN-96	Minnesota
4	*P. coronata*	*A. sterilis* L.	*R. palaestina* Boiss.	B	TA-8640	Israel, Mount Carmel
5	*P. graminis* Pers.: Pers. f. sp. *secalis* Eriks. & E. Henn.	*Agropyron repens* (L.) P. Beauv.	*Berberis vulgaris* L.	B,P	T90-21-1-AR	Minnesota
6	*P. graminis* Pers.: Pers. f. sp. *tritici* Eriks. & E. Henn.	*Triticum aestivum* L.	*B. vulgaris*	B	Race 29 (HJCS)	North Dakota
7	*P. g. tritici*	*T. aestivum*	*B. vulgaris*	B	Race 56 (MBCT)	North Dakota
8	*P. g. tritici*	*T. aestivum*	*B. vulgaris*	B	75-45-1772-1	Washington
9	*P. g. tritici*	*T. aestivum*	*B. vulgaris*	B	WW-1370	Minnesota
10	*P. g. tritici*	*T. aestivum*	*B. vulgaris*	U	74-36-924-A	Pennsylvania
11	*P. hordei* G. Otth	*Hordeum bulbosum* L.	*Ornithogalum* spp.	B	TA-5310	Israel, Judean foothills
12	*P. hordei*	*H. bulbosum*	*Ornithogalum* spp.	B	TA-5311	Israel, Judean foothills
13	*P. hordei*	*H. murinum* L.	Unknown[c]	B	TA-4135	Israel, Judean foothills
14	*P. hordei*	*H. spontaneum* C. Koch	*Ornithogalum* spp.	B	TA-1659	Israel, Judean foothills
15	*P. hordei*	*H. spontaneum*	*Ornithogalum* spp.	B	TA-1667	Israel, Judean foothills
16	*P. hordei*	*H. spontaneum*	*Ornithogalum* spp.	B	TA-1673	Israel, Judean foothills
17	*P. hordei*	*H. spontaneum*	*Ornithogalum* spp.	B	TA-1674	Israel, Judean foothills
18	*P. hordei*	*H. spontaneum*	*Ornithogalum* spp.	B	TA-1684	Israel, Judean foothills
19	*P. hordei*	*H. spontaneum*	*Ornithogalum* spp.	B	TA-1688	Israel, eastern upper Galilee
20	*P. hordei*	*H. spontaneum*	*Ornithogalum* spp.	B	TA-1690	Israel, Judean foothills
21	*P. hordei*	*H. vulgare* L.	*Ornithogalum* spp.	B	TA-860	Israel, southern coastal plain
22	*P. recondita* Roberge ex Desmaz.	*Aegilops longissima* Schw. & Muschl.	*Anchusa* spp.	B	TA-9310	Israel, central coastal plain
23	*P. recondita*	*A. longissima*	*Anchusa* spp.	B	TA-9311	Israel, central coastal plain
24	*P. recondita*	*A. ovata* L.	*Echium* spp.	B	TA-9295	Israel, western Samarian mountains
25	*P. recondita*	*A. sharonensis* Eig	*Anchusa* spp.	B	TA-9286	Israel, central coastal plain
26	*P. recondita*	*A. variabilis* Eig	*Anchusa* spp.	B	TA-9304	Israel, Judean foothills
27	*P. recondita*	*Triticum durum* Desf.	*Thalictrum speciosissimum* L.	B	TA-9314	Israel, central coastal plain
28	*P. sorghi* Schwein.	*Zea mays* L.	*Oxalis* spp.	B,U	See[d]	Minnesota
29	*Uromyces appendiculatus* (Pers.:Pers.) Unger	*Phaseolus vulgaris* L.	Autoecious	B,U	53	Minnesota
30	*U. reichertii* Anikst. & Wahl	*H. bulbosum*	*Scilla hyacinthoides* L.	B	TA-6315	Israel, Valley of Esdraelon
31	*U. vignae* Barclay	*Vigna unguiculata* L.	Autoecious	B	CPR-1	?

[a] B, basidiospore; P, pycniospore; U, urediniospore.
[b] Most of the regions designated in Israel correspond to regions listed by Eyal et al (9).
[c] Unknown in nature; *Ornithogalum* spp. are hosts when artificially inoculated.
[d] Collected from sweet corn, 1990, St. Paul, MN.

Oidia of *Coprinus cinereus,* strain P2301 B(a), were added to basidiospores to monitor consistency of staining procedures. Each oidium contained one haploid nucleus. The fungus was grown at 35 C on the medium of Rao and Niederpruem (21). Oidia were harvested 2–3 wk after inoculation.

In preparation for germination of teliospores, leaf pieces bearing telia were floated for 7–30 days on distilled water at 4 C as described by Anikster (2–4). For germination, the leaf pieces were placed on wet filter paper on the inside surface of lids of petri dishes. Glass slides were placed on wet filter paper on the bottom of each dish to receive basidiospores as they were ejected from promycelia (metabasidia) of germinating teliospores. Teliospores of most collections germinated within 1–4 days; a few required 10–15 days.

Slides were placed under actively sporulating telia and removed after 6 h. The basidiospores were located mostly within a 2-cm-diameter circle near the center of the slide. Basidiospores on slides were fixed at room temperature for 10 min in 50% ethanol and 12 h or more in 70% ethanol. The basidiospores adhered tightly to the slides throughout the fixing and staining procedures.

Pycniospores were placed on slides in drops of nectar obtained from pycnia. The nectar was allowed to dry before the spores were fixed. Urediniospores were placed on water droplets on slides, and the droplets were allowed to dry. Although many urediniospores were lost during fixing and staining, enough remained for measurement of nuclear fluorescence.

Coprinus oidia were harvested with a spatula and washed three times by suspending them in McIlvaine's buffer (24), pH 7, and centrifuging them at 700 *g*. The oidia were then fixed at 5 C in 70% ethanol and stored. Oidia in suspension were pipetted onto portions of slides that had previously been prepared with fixed basidiospores. The oidia suspension was allowed to dry on the slides.

Oidia and rust spores together on the slides were stained with PI (Sigma Chemical Co., St. Louis, MO) in combination with RNase treatment (Sigma) following the procedure of Tetsuka et al (25). PI, 1.5 μg/ml, was dissolved in NS buffer (16) containing 50 μg/ml RNase. The RNase stock solution (10 mg/ml) was boiled for 10 min to inactivate DNase.

About 0.1 ml of PI-RNase solution was pipetted onto the spore-bearing area of each slide and covered with a 22-mm-square coverslip. The slides were incubated at room temperature in a humid chamber for about 16 h. Coverslips were pressed lightly to squeeze out excess dye solution and then sealed to the slides with fingernail polish.

Intensity of nuclear fluorescence was measured with a Zeiss MPM OIK photometer (Carl Zeiss, Inc., Thornwood, NY) attached to a Zeiss photomicroscope III equipped for epifluorescence microscopy. A 100-W Hg lamp was used with Zeiss exciter/barrier filter set no. 14, which passed green excitation to the specimen and red emission to the viewer or photometer. Measurements were taken with a Zeiss Neofluor 25× objective lens, a 10× eyepiece, and Zeiss Optivar set at 1.6×, giving a total magnification of 400×. Excitation was applied through a 0.63-mm-diameter field aperture, which illuminated a field 50 μm in diameter. A measuring aperture, 0.4 mm in diameter, allowed light from a 10-μm-diameter field to reach the photometer during measurements.

The Zeiss PMI-2 indicator settings for the photometer were standardized using a fluorescent uranyl glass. With gain set at 100 and range set at 2, the "auto" was button-activated to obtain a reading of 100% transmittance. For measurement of nuclei, the range was set at 1, and neutral density filters that passed 30% of incident light were placed between the Hg lamp and the exciter filters. For *U. appendiculatus* only, filters passing 12% of the incident light were used and results adjusted accordingly. Fluorescence was reported in arbitrary units (AU), which were the values obtained from the photomultiplier output of the photometer as standardized with the uranyl glass.

Fluorescence was measured alternately for five to 10 rust spores and five oidia of *C. cinereus.* For each slide, fluorescence of 25–50 rust spores was usually measured; more spores were included if results were highly variable. For most rust collections, readings from 100 or more spores were obtained, but readings for only 50 were possible for some collections.

Primary data for each collection are given in histograms to show distribution of fluorescence intensities in the population of measured spores. The mean and standard deviation also are listed.

RESULTS

Because nuclei could not be seen with bright field microscopy, it was necessary to use epifluorescence to locate and align nuclei. Candidate basidiospores were examined for 1–2 s under epifluorescence to see whether the two nuclei of each spore were aligned side by side and not too far apart to be measured together (Fig. 1A). The optical system leading to the photometer was put in place, and the spore was aligned with the transmitted light with respect to the measuring aperture. Again the specimen was illuminated for 1–2 s to confirm alignment of the nuclei. The photometer was activated and a reading taken at about 1 s, when fluorescence intensity reached a peak before beginning to decline. Thereafter, fluorescence faded at a rate of 1% per second or less, an indication that the fading from the 2–4 s excitation during alignment was not a large source of variation in measured intensities.

PI-stained basidiospores had little background fluorescence compared with the intense fluorescence of nuclei (Fig. 1). However, a background reading was taken from an adjacent area (Fig. 1A) for each measurement of nuclear fluorescence. With an aperture large enough to encompass the two nuclei of a

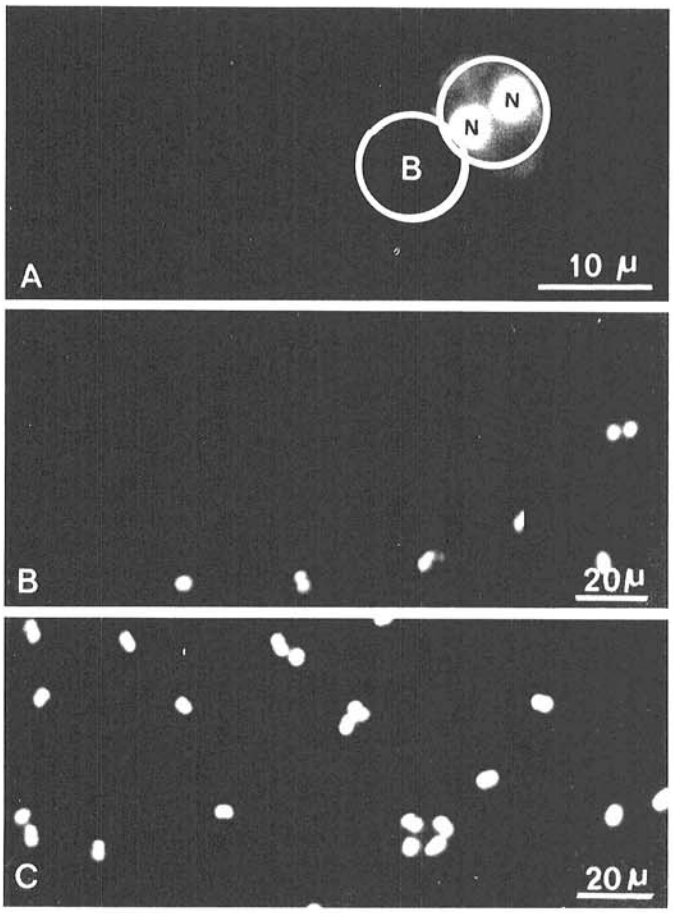

Fig. 1. Basidiospores stained with propidium iodide and viewed by epifluorescence microscopy. **A,** Basidiospore of *Puccinia hordei* (collection #16) showing location of aperture for measuring fluorescence of the two spore nuclei (N) and of background (B). **B,** *Uromyces appendiculatus* (collection #29); nuclei were more variable in shape and fluorescence intensity than nuclei of other species. **C,** *U. vignae* (collection #31).

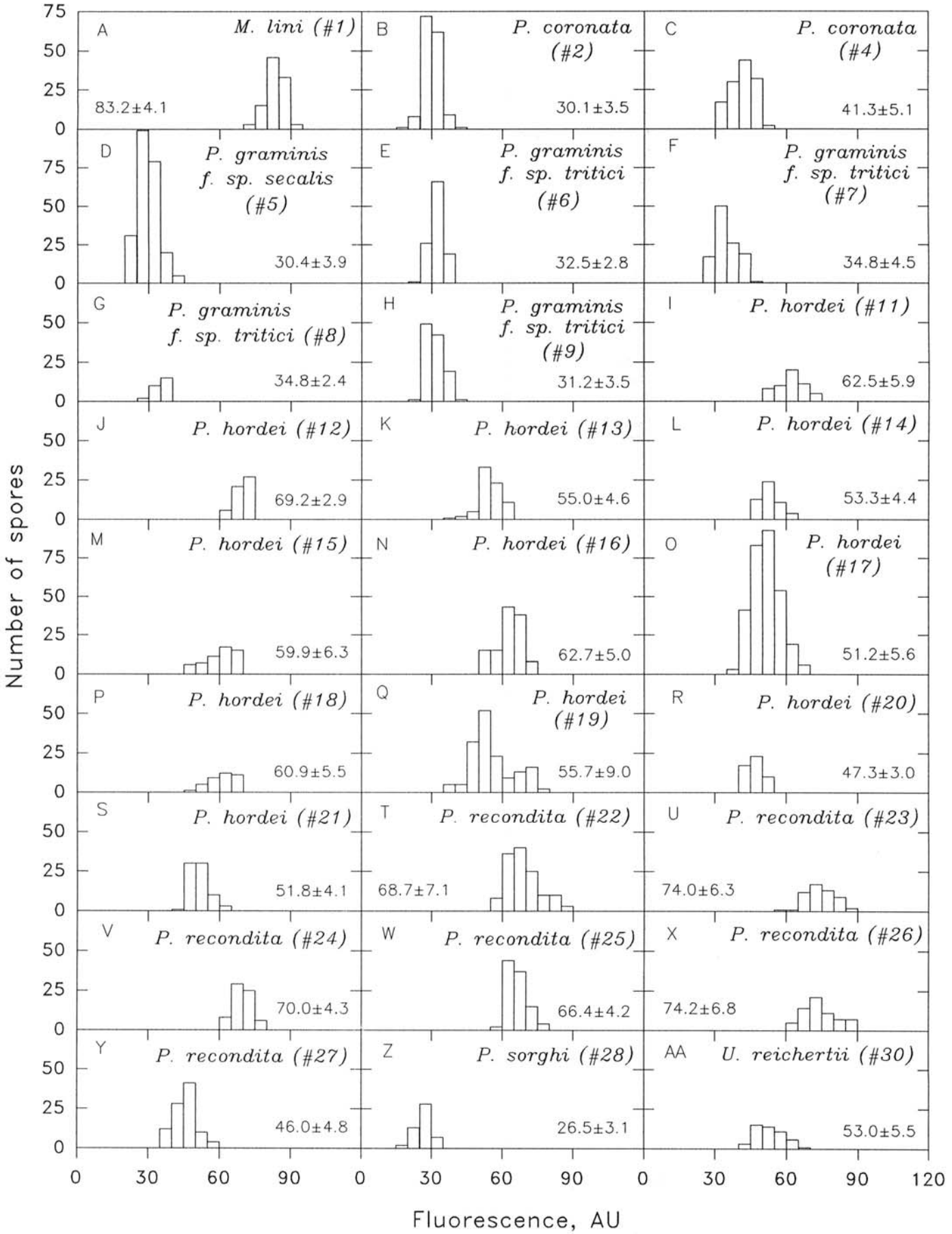

Fig. 2. Fluorescence intensity of propidium iodide-stained nuclei in basidiospores of *Melampsora lini,* five species of *Puccinia,* and *Uromyces reichertii.* Data are from the combined fluorescence of the two nuclei of each basidiospore. Collection numbers (in parentheses) are as listed in Table 1. The mean ± the standard deviation is given for each collection.

basidiospore, background measurements usually could not be taken within the confines of the spore. Background fluorescence was only 1–2 AU whether measured within or outside the basidiospore, compared with much higher values for the two nuclei (27–231 AU, depending on the species) (Figs. 2 and 3). Consequently, background readings were routinely taken from fields mostly outside basidiospores (Fig. 1A). The same was done with pycniospores that also had little or no background fluorescence. Urediniospores, on the other hand, were larger and had significant background fluorescence within spores. Therefore, background measurements for urediniospores were taken entirely within the spore except for *U. appendiculatus,* where nuclei were so large that background readings could be taken only partially within the spore. In all cases, the background reading was subtracted from the reading for the two measured nuclei.

The nuclei of *C. cinereus* that were measured on each slide concomitantly with rust nuclei had a mean fluorescence of 6.5 AU. Among slides, variation for *C. cinereus* did not correlate consistently with variation for basidiospores in a given collection (Table 2). Therefore, we did not use *C. cinereus* as a biological standard as initially planned. Instead we relied on the physical standard provided by the uranyl glass used to calibrate the photometer. In this way, we could not correct for possible staining variation among slide preparations. However, data for rust spores from a given slide were not used if the mean value for *C. cinereus* was outside the range of 5.5–8.0 AU or if the standard deviation exceeded 10% of the mean.

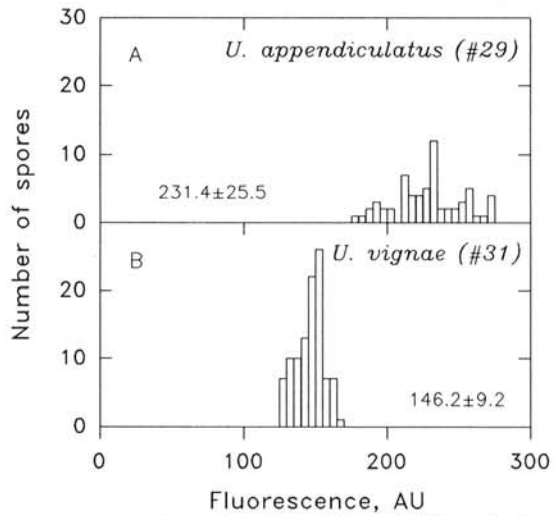

Fig. 3. Fluorescence intensity of propidium iodide-stained nuclei in basidiospores of *Uromyces appendiculatus* and *U. vignae.* Data are for the combined fluorescence of the two nuclei of each basidiospore. Collection numbers (in parentheses) are as listed in Table 1. The mean ± the standard deviation is given for each collection.

To learn whether DNA content of basidiospores changed with age, germinating teliospores of *P. hordei* (collection #17) were allowed to eject basidiospores onto slides for periods of 0.3–24 h (Table 2). The basidiospores were immediately fixed and stained with PI. Fluorescence intensity was the same for all harvest periods (Table 2), indicating that DNA content was constant. Consequently, we harvested basidiospores for 6 h in all other experiments.

The basidiospores of most species examined had two well-defined nuclei (Fig. 1), as is normal for rust fungi (3). Occasionally, a basidiospore appeared to have only one nucleus, probably because its nuclei were superimposed. In addition, some slides of collection #2 of *P. coronata* had a high proportion of basidiospores with 3–4 nuclei. Multiple nuclei also were seen occasionally in some spore clusters on portions of slides of *P. hordei* and *P. graminis.* Measurements of nuclear fluorescence were made only with basidiospores exhibiting two nuclei.

Histograms of fluorescence of basidiospore nuclei usually showed single peaks, indicating that the spore population was homogeneous for amount of nuclear DNA (Figs. 2 and 3). Mean values (listed in Figs. 2 and 3) were generally located close to the histogram peaks. However, collection #19 of *P. hordei* showed a small second peak at an intensity about 50% higher than that of the main peak (Fig. 2Q). Another exception was collection #29 of *U. appendiculatus,* which had no distinct peak and a wide variation in values (Fig. 3A). As viewed through the microscope, the nuclei in basidiospores of *U. appendiculatus* (Fig. 1B) had less distinct boundaries and were more irregular in shape, size, and apparent fluorescent intensity than nuclei in basidiospores of *U. vignae* (Fig. 1C) or other species observed.

Mean fluorescence intensity for basidiospores of the nine species measured spanned a ninefold range from 26.5 AU for *P. sorghi* to 231 AU for *U. appendiculatus* (Table 3). The lowest values were for *P. sorghi, P. graminis,* and *P. coronata,* whereas the other *Puccinia* species, *P. hordei* and *P. recondita,* had about twice those values. *U. reichertii* was intermediate with a value of 53 AU. *Melampsora lini* nuclei had a fluorescence intensity of 83.2 AU, higher than values for *Puccinia* spp. but less than values of *U. vignae* and *U. appendiculatus,* which had the highest values of all. The mean value for *U. appendiculatus* (231 AU) may have less accuracy than the values for the other fungi because of irregularities in nuclear configuration and the absence of a single distinct peak in the histogram, as noted above.

Within species, fluorescence of stained basidiospore nuclei showed variation related to the hosts from which telia were collected (Table 4). For *P. coronata,* a collection from *A. sativa* (#2) showed less fluorescence than a collection from *A. sterilis*

TABLE 2. Fluorescence of propidium iodide-stained nuclei of basidiospores of *Puccinia hordei*, collected from germinating teliospores for differing times, and of oidia of *Coprinus cinereus*

		P. hordei[a]		*C. cinereus*	
Slide	Duration of collecting (h)	Number of spores	Fluorescence[b] (mean ± SD)	Number of oidia	Fluorescence[c] (mean ± SD)
1	0.3	12	51.2 ± 2.2	10	7.2 ± 0.3
2	1.0	17	51.2 ± 3.2	12	7.1 ± 0.4
3	2.0	50	49.8 ± 3.4	19	6.3 ± 0.3
4	4.0	75	52.4 ± 4.4	25	6.0 ± 0.4
5	7.0	37	54.4 ± 3.8	10	6.2 ± 0.3
6	24.0	75	45.9 ± 4.4	31	6.4 ± 0.3
Mean			50.8 ± 2.6[d]	18	6.5 ± 0.4[d]

[a] Collection #17.
[b] Combined fluorescence for two nuclei per basidiospore measured in arbitrary units.
[c] Fluorescence of the single nucleus of each oidium measured in arbitrary units.
[d] Standard deviation based on variation of mean values among slides.

TABLE 3. Mean fluorescence intensity of propidium iodide-stained basidiospore nuclei from nine rust fungus species, listed in order of increasing fluorescence

Species	Number of collections[a]	Fluorescence[b,c]	Fluorescence relative to *Puccinia graminis*
P. sorghi	1	26.5 ± 3.1	0.8
P. graminis	5	33.7 ± 1.8	1.0
P. coronata	2	35.7 ± 5.6	1.1
Uromyces reichertii	1	53.0 ± 5.5	1.6
P. hordei	11	57.2 ± 6.0	1.7
P. recondita	6	66.5 ± 9.5	2.0
Melampsora lini	1	83.2 ± 4.1	2.5
U. vignae	1	146.2 ± 9.2	4.3
U. appendiculatus	1	231.0 ± 25.5	6.9

[a] See Figures 2 and 3 for data for individual collections.
[b] Combined fluorescence of two nuclei per basidiospore measured in arbitrary units.
[c] Mean ± standard deviation based on means of two or more collections or on means for individual nuclei where only one collection was measured.

(#4). For *P. hordei,* collections from *Hordeum bulbosum* (#11 and 12) had more fluorescence than collections from *H. murinum, H. spontaneum,* or *H. vulgare.* Finally, a collection of *P. recondita* from *Triticum durum* (#27) had less fluorescence than collections from four species of *Aegilops.* Where more than one collection was available for a given host species (*H. bulbosum* [#11 and 12] and *H. spontaneum* [#14–20] for *P. hordei, A. longissima* [#22 and 23] for *P. recondita*), variation among collections for each host species was not significant as judged from standard deviations (Fig. 2).

We measured the fluorescence of PI-stained nuclei of pycniospores of *P. coronata* and *P. graminis* (Fig. 4A and B), two species for which the spores were available. As expected, the pycniospores, which had one nucleus, exhibited about half the fluorescence of the corresponding basidiospores, which had two nuclei. Thus, for *P. coronata,* pycniospores had 17.9 AU (Fig. 4A) and basidiospores had 30.1 AU (Fig. 2B). Likewise, for *P. g. secalis,* pycniospores had 15.8 AU (Fig. 4B) and basidiospores had 30.4 AU (Fig. 2D). These results indicate that, for each species, the nuclei of both spore types were the same in DNA content.

TABLE 4. Mean fluorescence intensity of propidium iodide-stained basidiospore nuclei of collections grouped by host for *Puccinia coronata, P. hordei,* and *P. recondita*

Species	Host	Designation of collection[a]	Number of spores	Fluorescence[b]
P. coronata	*Avena sativa*	2	153	30.1 ± 3.5[c]
	A. sterilis	4	125	41.3 ± 5.1
P. hordei	*Hordeum bulbosum*	11,12	108	65.8 ± 3.3[c]
	H. murinum	13	75	55.0 ± 4.6
	H. spontaneum	14–20	770	55.7 ± 4.9
	H. vulgare	21	74	51.8 ± 4.1
P. recondita	*Aegilops longissima*	22,23	186	71.3 ± 2.6
	A. ovata	24	68	70.0 ± 4.3
	A. sharonensis	25	102	66.4 ± 4.2
	A. variabilis	26	65	74.2 ± 6.8
	Triticum durum	27	95	46.0 ± 4.8[c]

[a] See Table 1 for identification of collections.
[b] See Figure 2 for data for individual collections. Mean ± standard deviation based on values of individual measurements. Combined fluorescence (measured in arbitrary units) of two nuclei per basidiospore.
[c] Significantly different from values for other hosts within the species by Student's t test. $P = 0.01$.

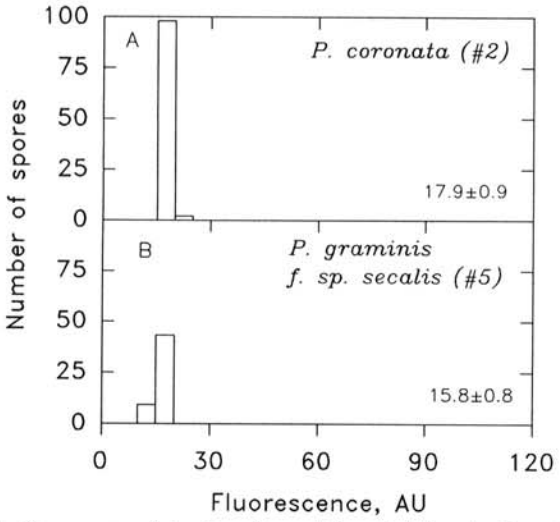

Fig. 4. Fluorescence intensity of propidium iodide-stained nuclei in pycniospores of *Puccinia coronata* and *P. graminis* f. sp. *secalis.* Data are for the single nucleus of each spore. Collection numbers (in parentheses) are as listed in Table 1. The mean ± the standard deviation is given for each collection.

To compare basidiospores with urediniospores in DNA content, we chose two species that had given satisfactory results from basidiospores, *P. coronata* and *P. graminis* f. sp. *tritici,* and one that had given less satisfactory results, *U. appendiculatus.* The urediniospores (Fig. 5) varied more than did basidiospores in nuclear configuration and in intensity of background fluorescence. Therefore, we selected for measurement urediniospores that had relatively compact nuclei and low background fluorescence. After subtraction of background, the combined fluorescence of the paired nuclei in urediniospores equaled that from paired nuclei in basidiospores in both *P. coronata* and *P. g. tritici.* For *P. coronata* from *Avena sativa,* urediniospores had 37.4 AU (Fig. 6A) and basidiospores had 30.1 AU (Fig. 2B). Likewise, for *P. g. tritici,* urediniospores had 34.0 AU (Fig. 6A) and basidiospores had 31.2–34.8 AU (Fig. 2E–H). For *U. appendiculatus,* on the other hand, the fluorescence of the two nuclei in urediniospores was only 91.4 AU (Fig. 6C), compared with 231.4 AU for the two nuclei in basidiospores (Fig. 3A). On the basis of the values from urediniospores, the fluorescence for *U. appendiculatus* (91.4 AU) was 2.7 times that obtained for *P. graminis* (34.0 AU) (Fig. 6B).

DISCUSSION

With the assumptions that basidiospores of diverse rust fungi are haploid and in the same stage of the nuclear cycle, the fluorescence of PI-stained nuclei can be used to estimate relative nuclear DNA content among species. Quantitative estimates of relative DNA content with PI-stained nuclei have correlated closely with results using Feulgen-based staining methods in animals (10,11) and in diverse plant species (19). Nuclear fluorescence intensities of individual rust species relative to *P. graminis* are listed in Table 3. *P. sorghi, P. graminis,* and *P.*

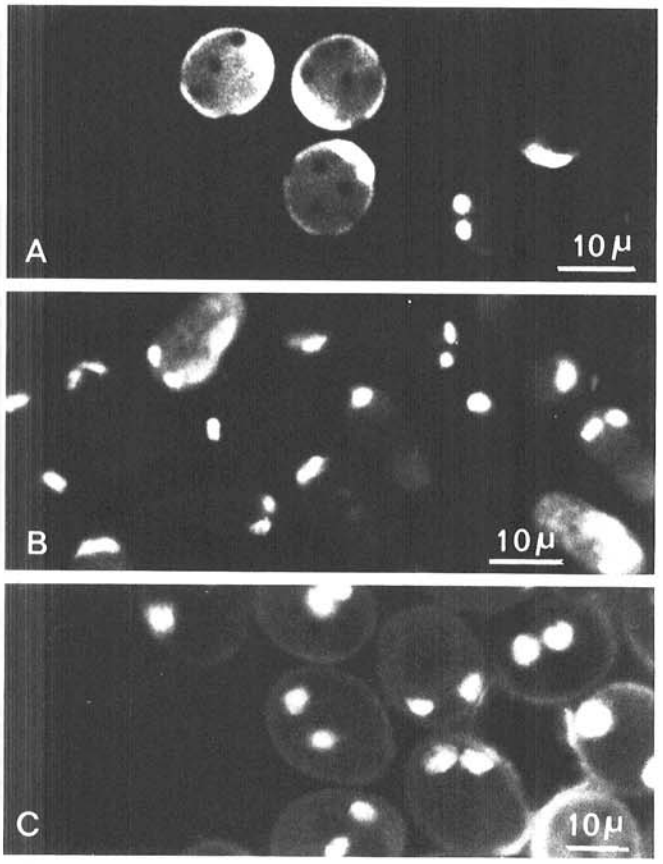

Fig. 5. Urediniospores stained with propidium iodide and viewed by epifluorescence microscopy. **A,** *Puccinia coronata* (collection #3); **B,** *P. graminis* f. sp. *tritici* (collection #10); C, *Uromyces appendiculatus* (collection #29).

coronata formed a group with relatively low DNA content. Of the species investigated, this group contains the best candidates for genetic mapping where small genomes are advantageous. The other species had DNA content ranging from 1.6 times that of *P. graminis* for *U. reichertii* to 4.3 for *U. vignae*. These ratios can be used to estimate absolute DNA content, because the genome of *P. g. tritici* is known to contain 53×10^6 base pairs (6). Likewise, *P. sorghi* contains 43×10^6 base pairs (1) or 0.8 times that of *P. graminis,* equal to the ratio we obtained for nuclear fluorescence of the two species (Table 3).

An extremely high mean value was obtained for fluorescence intensity of nuclei in basidiospores of *U. appendiculatus,* 6.9 times the value for *P. graminis* (Fig. 3 and Table 3). The nuclei were not well defined and the histogram of fluorescence intensities showed no distinct peak. On the basis of data for urediniospores, which gave clear-cut histograms, the ratio for fluorescence of *U. appendiculatus* to that of *P. graminis* was 2.7 (Fig. 5B and C). However, data obtained recently for pycniospores using flow cytometry gave a ratio of 9.1 (data not shown), confirming the results here with basidiospores. *U. appendiculatus* has an unusually large amount of DNA per nucleus.

As part of an investigation of DNA content of rust mycelium grown in axenic culture and in urediniospores, Tetsuka et al (25) obtained values for PI-stained nuclei that indicated that *P. g. tritici* and *P. coronata* were similar in DNA content, which agrees with our results. Similarly, Wittmann-Meixner (27), working with DAPI-stained nuclei, apparently in germ tubes of urediniospores, showed that *P. sorghi* was similar to *P. graminis* in DNA content and that *U. appendiculatus* had more than twice as much DNA as did *P. graminis,* again in agreement with our results (Table 3 and Fig. 5). In contrast, both Tetsuka et al (25) and Wittmann-Meixner (27) reported that *P. recondita* equaled *P. graminis* in DNA content, whereas we found twice as much in *P. recondita.*

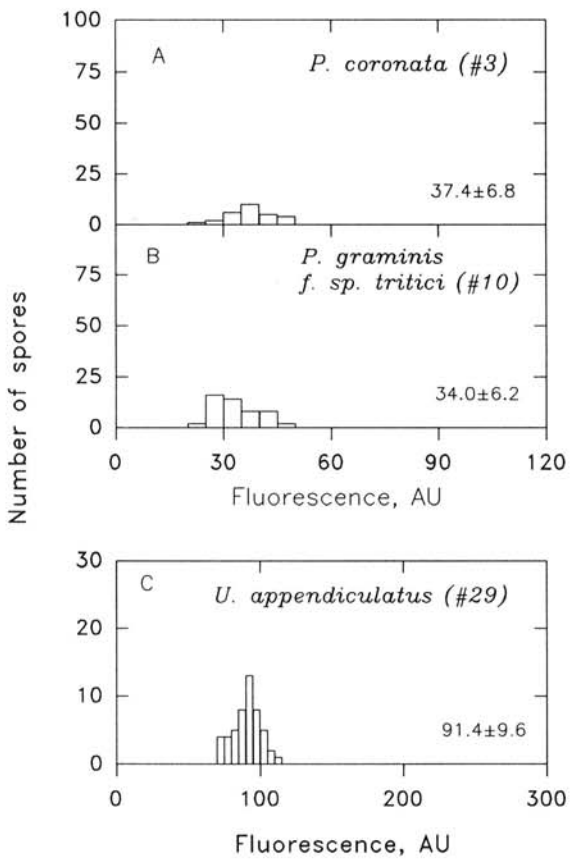

Fig. 6. Fluorescence of propidium iodide-stained nuclei in urediniospores of *Puccinia coronata, P. graminis* f. sp. *tritici,* and *Uromyces appendiculatus.* Data are for the combined fluorescence of the two nuclei of each urediniospore. Collection numbers (in parentheses) are as listed in Table 1. The mean ± the standard deviation is given for each collection.

Furthermore, Wittmann-Meixner's data (27) indicate that an isolate of *P. coronata* had half the DNA content of *P. graminis,* whereas we found the two species to be equal. As noted earlier, the DAPI stain used as fluorochrome by Wittmann-Meixner (27) is less reliable than PI for comparing species because it binds to AT-rich regions of DNA, which vary in amount among species. Nevertheless, the inconsistencies in results may relate in some cases to differences in DNA content within species.

For *P. recondita,* the collections of Tetsuka et al (25) and Wittmann-Meixner (27) were from *T. aestivum,* whereas ours were from either *T. durum* or *Aegilops* (four species) (Table 1). Furthermore, the collections we used from *Aegilops* all had more DNA than did a collection from *T. durum* (Table 4). *P. recondita* is a complex and diverse species with respect to telial host, alternate host, geographical origin, and other factors (7,14,22). Possible differences in DNA content need to be determined more completely to help establish relationships within this species.

P. coronata also may have variations in DNA content within the species. The average for all collections was approximately equal to that of *P. graminis,* but basidiospores of collection #2 from *A. sativa* had only 75–80% of the DNA content of basidiospores of collection #4 from *A. sterilis* (Fig. 2) or urediniospores of collection #3 from *A. sativa* (Fig. 6). Thus, we did not find a collection of *P. coronata* with DNA content relative to *P. graminis* as low as that of Wittmann-Meixner (27). Nevertheless, a more comprehensive sampling is required to determine patterns of variation within *P. coronata.*

Basidiospores of a collection of *P. hordei* from *H. bulbosum* had about 120% of the DNA content of basidiospores of *P. hordei* from other hosts (Table 4). Collections from *H. bulbosum* differ from others in telial host range, in alternate host, and in ability to hybridize in nature with collections from other hosts. Collections from *H. bulbosum* may represent a distinct forma specialis (5,8). Again, a more comprehensive sampling is needed to determine if collections from *H. bulbosum* consistently have more DNA than collections from other host species.

Basidiospores of most species proved well suited for determination of relative nuclear DNA content in our investigation. Background fluorescence was minimal, and histograms indicated spore populations were homogeneous with respect to DNA content. Only *U. appendiculatus* failed to yield a histogram with a distinct peak, as noted earlier. Furthermore, DNA amounts were constant for 24 h after basidiospores were ejected from metabasidia. Pycniospores were well suited for measurement of nuclear DNA content in the two species we used, *P. coronata* and *P. graminis.* Spores contained single, well-defined, brightly fluorescent nuclei that were uniform in intensity within spore populations (Fig. 4).

In urediniospores, the net intensities of nuclear fluorescence after subtraction of background in *P. graminis* and *P. coronata* (Fig. 6) were close to the values obtained for basidiospores of the same species (Fig. 2). Furthermore, for *P. g. tritici,* values for fully pigmented urediniospores were virtually the same as values for an orange mutant lacking wall pigment or a white mutant lacking both wall and cytoplasmic pigment (data not shown). Apparently, pigmentation does not interfere significantly with emission of fluorescence by PI-stained nuclei in urediniospores. Nevertheless, variations in nuclear configuration and in background intensity make urediniospores less suitable than basidiospores for determination of nuclear DNA content.

The amount of fluorescence in individual PI-stained nuclei was the same in basidiospores, pycniospores, and urediniospores in *P. coronata* (Figs. 2B and C, 4A, and 6A). This was also indicated for single nuclei of *P. graminis,* where values for pycniospores (Fig. 4) equaled those for basidiospores for a collection (#5) of *P. g. secalis* (Fig. 2D) that, in turn, equaled values from urediniospores of a collection (#10) of *P. g. tritici* (Fig. 6B). Yamazaki and Katsuya (29) likewise found equal PI fluorescence in nuclei of basidiospores, pycniospores, and urediniospores, and also in aeciospores, of *Cronartium quercuum.* Thus, the available data indicate that all spores with haploid nuclei in the rust life cycle have the same DNA content for a given species. However, Hu (12),

measuring fluorescence of DAPI-stained nuclei in *C. quercuum* f. sp. *fusiforme,* found that the DNA content of nuclei was higher in basidiospores than in hyphae grown axenically from them. She suggested that DNA content had doubled in basidiospores in preparation for the next mitotic nuclear division, i.e., that the nuclei were in the G2 phase of the cell cycle. Likewise, Kwon and Hoch (17) provided strong evidence that urediniospores of *U. appendiculatus* are in G2. Together, the evidence suggests that rust spores with haploid nuclei (all spores except teliospores) are in G2, a possibility that needs to be investigated with additional comparisons between spores and hyphae.

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