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TWO NEW HYBRID *DENDROICA* WARBLERS AND NEW METHODOLOGY FOR INFERRING PARENTAL SPECIES

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ABSTRACT.—A phenotypic hybrid between *Dendroica townsendi* × *D. nigrescens* is described from coastal British Columbia. It carried the mitochondrial-DNA (mtDNA) haplotype of *nigrescens*, indicating a *nigrescens* mother in the original cross that led to this hybrid. This specimen is used to demonstrate the special value of "contradictory characters" in refuting alternate hypotheses of parentage for hybrids. Contradictory characters exist when a potential hybrid exhibits character states more extreme than those found in both of a possible pair of parents. While intermediate characters can suggest a hybrid ancestry, intermediate character states may also be consistent with a variety of potential pairs of species as parents, a point I demonstrate using this *townsendi* × *nigrescens* hybrid. Phenotypically, the second specimen is a typical *townsendi*, but its mtDNA haplotype was that of *virens*. An extensive survey of *townsendi* haplotypes revealed no other polymorphism for the *virens* haplotype; furthermore, at least four mutations would be required to move from a *townsendi* to a *virens* haplotype. Together these observations suggest that this specimen is a highly backcrossed descendent of a *townsendi* × *virens* cross, a hypothesis consistent with the fact that this specimen was collected where the ranges of *townsendi* and *virens* very nearly make contact in the Rocky Mountains of British Columbia. *Received 9 November 1992, accepted 22 December 1992.*

MOST REPORTS of unusual hybrids are cast in the general context of telling us something about relationships, either confirming current ideas or raising new possibilities. A cladist, however, views interbreeding as an ancestral condition; thus, in a cladistic analysis, the absence of interbreeding between sympatric species is important as a derived character or "condition," but interbreeding offers only a vague notion of relatedness (McKitrick and Zink 1988). In a larger species swarm, a hybrid between two particular species only tells us that genomes are compatible enough to produce a viable offspring, and not that the hybridizing taxa are sisters. Without systematic experimental crosses, such knowledge is of limited usefulness in developing phylogenies. If so, then why should the occasional natural hybrid be reported at all?

Surely the most fundamental answer is that odd specimens demand explanation, and a hybrid origin is one of many possibilities. Where the character states of several species suggest their possibility as parents, reliably inferring parentage in the absence of a direct observation of interbreeding may be difficult. Here I develop two criteria for doing so. The first treats intermediacy in characters that distinguish potential pairs of parents. However, as I show below, the analysis of intermediate characters may fail definitively to identify the parents. The second criterion treats character states in the hybrid that contradict it being a product of two hypothetical parental species. This arises when hypothetical parents either share a trait that is not found in the putative hybrid or when hypothetical parents differ in their states for a character but the puted hybrid is more extreme in that character than either of the parents. Taken together, the analyses of potentially intermediate and potentially contradictory characters suggest parentage rather convincingly.

Apart from their intrinsic interest, descriptions of hybrids have other important values. For example, even a low frequency of interbreeding can result in gene exchange. When biochemical characters such as isozymes or mitochondrial-DNA (mtDNA) fragment polymorphisms are used to develop phylogenies or deduce population structure, shared genetic material could be difficult to explain without having available the knowledge that viable hybrids can be produced between the species in question.

Knowledge of the distribution and frequency of hybrids within a clade may also challenge existing classifications. In this case hybrids pose several questions that are best addressed by systematic revisions based upon characters that were not used to produce the earlier classifications. Is the earlier classification, interpreted as a phylogeny, correct (e.g. Short 1969; but see Rosen 1979, Cracraft 1983)? When intergeneric hybrids outnumber intrageneric hybrids, is this because selection acts to develop isolating mechanisms between closely related species, but not between distantly related species that, for many reasons, will rarely interbreed (Parkes 1961, Banks and Johnson 1961)? Do hybrids between nonsister taxa occur primarily at the edges of ranges, where individuals of the uncommon species have difficulty finding conspecific mates (Short 1969)? Rohwer (1972) provided an example of geographic variation in the incidence of hybridization apparently related to the difficulty of an uncommon species finding conspecific mates.

The expression of characters in hybrids may also offer insight into the minimum number of different gene complexes that control what, by morphology, appears to be a single trait. A single morphological difference between the two parental types can sometimes be seen to recombine in hybrids in ways that show it to be controlled by two or more separate complexes of genes. For example, flank streaks are absent in *Dendroica occidentalis* and well developed in *D. townsendi*, giving the impression of a single character. But flank streaks in hybrids between these two species are sometimes pronounced anteriorly but absent or reduced posteriorly, or vice versa; thus, at least two partially independent gene complexes control this single morphological trait (Rohwer and Wood unpubl. data). This supposition is confirmed by the fact that in *virens* the posterior flanks are streaked as in *townsendi*, but the anterior flanks are solid black, becoming lateral extensions of the bib. In the *virens* species complex the derived character, "fused streaks," is found only in *virens*, but hybrids between *townsendi* and *occidentalis* suggest that the genetic potential for this development existed in the shared common ancestor of these three species.

In this paper, I describe the first known hybrid between *D. townsendi* and *D. nigrescens*. This specimen is intermediate in plumage between *townsendi* and *nigrescens*, and is used to illustrate the value of analyzing both intermediate and "contradictory" characters to infer parentage. The mtDNA haplotype of this hybrid matches that of *nigrescens*. A second specimen matches *townsendi* in all of its plumage characters and, therefore, is not a morphological hybrid. However, the latter specimen carries the mtDNA haplotype of *virens* and may have descended from a *townsendi* × *virens* cross.

METHODS

Both specimens were obtained in the course of collecting specimens for use in a study of hybridization between *townsendi* and *occidentalis*. The *townsendi* × *nigrescens* hybrid (SAR 5629) was collected on 1 June 1988, 12.1 km northeast of Gold River, Vancouver Island, British Columbia. It was attracted to the playback of *townsendi* song; the bird sang like *townsendi* in this region and not at all like *nigrescens*.

The *townsendi* with a *virens* mtDNA haplotype (CSW 3603) was collected on 17 June 1988 in eastern British Columbia, 5 km west, 9 km south of Valemount. It was taken in mature second-growth Douglas fir (*Pseudotsuga menziesii*) forest where *townsendi* were common.

Within a few minutes of being collected, each of these specimens was buried in crushed ice for rapid cooling. They were skinned 1 to 3 h later, at which time tissue samples were removed and placed either on dry ice or into liquid nitrogen. Upon returning to the laboratory, these tissues were stored at −80°C until analysis. Genomic DNA was extracted and digested with three six-base-pair restriction enzymes (*Hind* III, *Xba* I, and *Bam* HI) following supplier recommendations. Fragments were separated by agarose gel electrophoresis with a molecular size standard. The mtDNA fragments were visualized by Southern hybridization with radio-labeled probes made from purified mtDNA of *townsendi* and *occidentalis* (for details on methods, see Shields and Helm-Bychowski 1988).

The *D. virens* species group is an assumed clade of five closely related species (*D. virens, townsendi, nigrescens, chrysoparia* and *occidentalis*) postulated to have arisen as a consequence of isolation in eastern and western forest refugia during successive Pleistocene glacial advances (Mengel 1964, 1970; but see Bermingham et al. 1992). Although the breeding range of *chrysoparia* is well isolated from other members of this clade, I have included it to complete the analysis of character states and better illustrate the value of contradictory character states in inferring parentage.

By comparing adult males of these five species, I found 19 plumage color and pattern characters that distinguished one or more pairs of species in this group (Table 1). Undoubtedly, these are not all independent characters; however, the recombinant plumages that would be found if more hybrid specimens were available would probably also reveal that some of these characters should be further subdivided. Expression of some of the 19 characters varies somewhat between second-year (SY) and after-second-year (ASY) males. (SY indicates second calendar year, which for summer birds in North Temperate regions would be their first potential breeding season;

TABLE 1. States for an SY hybrid (*D. nigrescens* × *D. townsendi*) 19 plumage characters that vary among ASY breeding males for the five species of the *Dendroica virens* complex.[a]

Character	Hybrid	*Dendroica*				
		nigrescens	*townsendi*	*occidentalis*	*virens*	*chrysoparia*
1 Face patch	Distinct	Distinct	Distinct	None	Faint	None
2 Color of face patch	Black	Black	Black	(Yellow)	Green	(Yellow)
3 Eye line posterior to eye	Distinct	Distinct	Distinct	None	Faint	Distinct
4 Color of eye line	Black	Black	Black	(Yellow)	Blackish-green	Black
5 Eye line from eye to bill	Complete	Complete	Complete	None	Complete	Broken
6 Superciliary stripe	Complete	Broken with black	Complete	(Complete)	Complete	Complete
7 Posterior portion of superciliary stripe	Pale yellow	White	Yellow	(Yellow)	Yellow	(Yellow)
8 Subciliary stripe	Pale yellow	White	Yellow	Yellow	Yellow	Yellow
9 Area posterior to face patch	Yellow tinge	White	Yellow	Yellow	Yellow	Yellow
10 Anterior crown	Black	Black	Black	Yellow	Green	Black
11 Posterior crown	Black[b]	Black	Black	Black	Green	Black
12 Nape	Gray	Gray	Green	Dark gray	Green	Black
13 Background of back	Greenish gray	Gray	Green	Gray/green	Green	Black
14 Back streaking	Moderate	Moderate	Moderate	Extensive	None or limited	None (black) or extensive
15 Extent of bib	Moderate	Moderate	Moderate	Limited	Extensive	Moderate
16 Anterior flanks	Streaked	Streaked	Streaked	White	Black	Streaked
17 Posterior flanks	Streaked	Streaked	Streaked	White	Streaked	Streaked
18 Breast	Pale yellow	White	Yellow	White	Yellow tinge	White
19 Lowermost flanks	White	White	White	White	Yellow	White

[a] If character does not exist (e.g. a contrasting "face patch"), I placed state of character in parentheses.

[b] Although posterior crown was blackish in this SY hybrid, I scored it as black because the area becomes darker in ASY individuals of both *nigrescens* and *townsendi*.

ASY birds are at least in their second potential breeding season.) For example, the back streaking is less well developed in SY than ASY *townsendi*, and the posterior crown may be black in ASY males but green or gray in SY males of *townsendi* and *nigrescens* (Pyle et al. 1987, Jackson et al. 1992, Rohwer and Wood unpubl. data). For this analysis of potential parents, I have listed ASY character states in Table 1. For the *townsendi* × *nigrescens* hybrid, which was an SY bird, I have scored age-variable characters such that a minimum number of intermediate or contradictory states was produced for the pairwise comparisons of Table 2; this convention changed the hybrid's score only on posterior crown (from blackish to black).

Some character states are given in parentheses in Table 1. I did this to alert the reader to the dependency between pattern and color characters when certain species pairs are being compared. For example, *occidentalis* and *chrysoparia* lack the face patches present in the other species of this clade. In the others, *virens* has a very small greenish face patch, whereas *townsendi* and *nigrescens* have large black face patches. Thus, in comparing *townsendi* and *occidentalis* characters 1 and 2 are equivalent, but in comparing *townsendi* and *virens* characters 1 and 2 are different.

I have not summarized in Table 1 whether or not intermediate character states would have been recognizable in hybrids between all potential pairs of parents. Yet, knowing whether intermediate states could be recognized has a substantial effect on counting the number of characters that could assume an intermediate state in a hybrid. For example, the difference between no face patch and a faint face patch is small, and an intermediate state might be difficult to recognize, especially without correcting for age-class variation (Table 1, character 1). However, a face patch intermediate between the faint and distinct categories would be easy to recognize, either because it was intermediate in size or because it was composed of a mix of feathers of different colors. Such information was considered when scoring characters for potential intermediacy in hybrids.

RESULTS

Analysis of intermediate character states.—When character differences are controlled by multiple alleles and inheritance is additive, hybrids should be intermediate between potential par-

TABLE 2. Summary of fraction of characters that are either intermediate or contradictory in hybrid compared to each of 10 possible parental combinations. Fractions above diagonal indicate number of intermediate characters/number of characters that could be intermediate between potential parents. Fractions below diagonal indicate number of contradictory characters/number of characters that could be contradictory between potential parents.

Dendroica species	*occidentalis*	*townsendi*	*virens*	*nigrescens*	*chrysoparia*
occidentalis	—	2/9	4/7	4/9	1/5
townsendi	5/11	—	1/6	5/5	2/6
virens	10/13	5/11	—	4/9	2/6
nigrescens	1/10	0/14	1/13	—	3/6
chrysoparia	9/16	4/17	7/16	5/17	—

ents. Even with differences produced by single loci, hybrids should rarely exhibit more extreme character states than those found in the parents. Thus, when the character states for potential parents differ sufficiently for intermediate states to be recognized, hybrids should be intermediate.

Of the 19 plumage characters listed in Table 1, *townsendi* and *nigrescens* may be distinguished by just seven characters; whether or not all are under separate genetic control is questionable. For example, I have listed the color of the posterior portion of the superciliary stripe, the color of the subciliary stripe, and the color of the area behind the face patch as three characters. These may well represent only a single character, but I have separated them because the intensity of yellow varies among them in the hybrid specimen (Table 1). Indeed, the only pattern difference between *townsendi* and *nigrescens* is the broken anterior portion of the eye line, which creates the yellow loral spot in *nigrescens*. Except for the loral spot, *nigrescens* is simply white on all parts of its face and breast where *townsendi* is yellow. The only other major difference between *townsendi* and *nigrescens* is in the background color of the back, where *nigrescens* is gray and *townsendi* is green. Only character variation among the five members of this group and variation in the appearance of these characters in the hybrid justifies dividing these two general color differences into seven characters.

The hybrid specimen is intermediate between *townsendi* and *nigrescens* in all five of the characters that distinguish this parental pair by two or more steps (Table 1: characters 7,8,9,13, and 18). Only for these characters could the hybrid be scored as intermediate on a character-by-character basis. Thus, the above-diagonal element for the *townsendi* × *nigrescens* cell of Table 2 reads 5/5, meaning that the specimen scored intermediate between these two potential parents for each of the five characters for which intermediate states could be recognized. For the two characters by which *townsendi* and *nigrescens* are separated by only a single step, the hybrid resembles *townsendi* in character 6 and *nigrescens* in character 12 (Table 1). For both characters the two extreme states seemed too similar for more than two-state scoring, so I treated each as two-state characters for this species pair.

Despite this strong confirmation of the hybrid's intermediate state between *townsendi* and *nigrescens*, the above-diagonal summary of intermediate character states in Table 2 reveals other plausible parental pairs, especially if the possibility of the specimen being backcrossed to one of the parental lineages is admitted. The specimen is intermediate on at least one character separating every potential pair of parents and it is intermediate on a third or more of the potentially intermediate characters separating all but two of the 10 potential pairs of parental species (Table 2). The important general point here is that, although intermediate character states are often the first to suggest a possible hybrid ancestry, they fail to provide anything more than a majority-rule vote on parentage. Any analysis of intermediacy will fail to exclude potential pairs of parents.

Analysis of contradictory characters.—Contradictory character states arise in two ways. First, the potential parents could have identical states for a character, while the hybrid exhibits some alternative state for that character. Second, the potential parents could differ in a character having ordered states, while the state for this character in the hybrid lies outside the range defined by the potential parents. The number of characters by which a hybrid could contradict and, thus, exclude any particular parentage, is the number of characters available, minus all characters by which a potential pair of parents do not display the entire range of states possible for those characters. Any unordered character must be treated as potentially contradictory because end-point character states are not definable. This figure is the denominator for the fractions in the below-diagonal half of Table 2. An analysis of contradictory character states is particularly useful when species differences are subtle, thus making the recognition of three or more character states and hybrid intermediacy in any single character impossible.

For the specimen in question, only a *townsendi* × *nigrescens* parentage fails to be contradicted by even a single character. For the 19 characters, 14 could have assumed contradictory states in a hybrid between *townsendi* and *nigrescens* because more extreme states were realized by other members of the group being analyzed. Not one of these 14 characters excludes a *townsendi* × *nigrescens* ancestry. At least four characters refute most other species pairs as potential parents. Only one character, however, refutes a *nigrescens* cross with either *occidentalis* or *virens* (Table 2). For *nigrescens* × *occidentalis*, a reasonable combination on geographic grounds, the yellow wash on the breast of the hybrid refutes them as parents because both have white breasts. For *nigrescens* × *virens*, an unreasonable combination on geographic grounds, the breast of the hybrid is considerably yellower than *virens* adults having the most extensive yellow tingeing on their breasts. It bears recognizing, however, that a *townsendi* × *nigrescens* hybrid that was moderately backcrossed to *nigrescens* might not refute a *nigrescens* × *virens* parentage because of the similarities between *virens* and *townsendi*.

To summarize, only for *townsendi* × *nigrescens* are there no contradictory character states (Table 2). Had this specimen been collected in the nonbreeding season, only this analysis of potentially contradictory character states would have led to a strong conclusion that it was indeed a *townsendi* × *nigrescens* hybrid. This is so because there are several other possible hybrid combinations in this species group that could not have been ruled out on geographic grounds. Thus, even if the ranges of these species were unknown, the analysis of contradictory character states would have excluded all of the nine other potential parental combinations.

mtDNA differences.—As part of other work on warblers of this complex, we have characterized the mtDNA of *nigrescens*, *virens*, *townsendi*, and *occidentalis* (Bermingham et al. 1992). Unfortunately, tissues for *chrysoparia* were unavailable for that study, but the geographic restriction of *chrysoparia* as a breeding bird to the Edwards Plateau of Texas makes omission of this species unimportant to the current analysis. The mtDNA haplotypes serve three purposes in this paper. First, because mtDNA is maternally inherited as a single "gene," it can be used to identify which species the mother belonged to in the original cross. Second, in doing so, it also confirms one of the species identities inferred by the plumage analysis for the *townsendi* × *nigrescens* hybrid. And third, it is only through the analysis of mtDNA haplotypes that the second specimen can be determined with very high probability to be a matrilineal descendant of a *townsendi* × *virens* cross. Table 3 provides the haplotype summaries for these two individuals and for many other specimens that were evaluated with the three restriction enzymes, *Hind* III, *Xba* I, and *Bam* HI.

In the putative *townsendi* × *nigrescens* hybrid, fragment patterns at all three restriction sites exclude *occidentalis* as the mother; *Xba* I and *Bam* HI both exclude *townsendi* as the mother, and *Bam* HI excludes a *virens* mother. *Dendroica virens* is only one or two restriction-site losses removed from *nigrescens* with *Bam* HI, depending on which of two *virens* haplotypes is considered. However, the plumage analysis and collecting locality both exclude *virens* as the mother. Therefore, this specimen must be either the F_1 progeny or a lineal descendant of a hybrid pairing between a female *nigrescens* and a male *townsendi* (Table 3).

The second specimen of interest is, by its plumage characters, a pure ASY male *townsendi*. However, its mtDNA haplotype matches that of *virens*, suggesting that it may be a lineal descendant of a cross between a female *virens* and a male *townsendi*. *Hind* III excludes *occidentalis* from the maternal lineage, a trivial exclusion because this bird was collected in the breeding season in the Rocky Mountains of eastern British Columbia, and the nearest breeding populations of *occidentalis* are in the Cascades of Washington and Oregon. *Bam* HI excludes *nigrescens* from the maternal lineage, another trivial exclusion because *nigrescens* just enters coastal British Columbia (for range maps of members

TABLE 3. Summary of mtDNA haplotypes observed for four potential parents and two putative hybrids with three restriction endonucleases.[a]

Species or individual	*Hind* III	*Xba* I	*Bam* HI
townsendi	B or E	T or S	Q, W or P
nigrescens	B	R	R
virens	B	R or Q	N or O
occidentalis	E	S	P
townsendi × *nigrescens*	B	R	R
townsendi with *virens* haplotype	B	R	O

[a] Different letters represent different haplotypes.

of *D. virens* species complex, see Morsé 1989:fig. I-3). Finally, *Xba* I and *Bam* HI exclude *townsendi* and *occidentalis* from its maternal lineage. Thus, *virens* is the only member of this species group that fails to be excluded from the maternal lineage by these three restriction enzymes.

The purported hybrid origin of this phenotypically *townsendi* specimen may be challenged on two grounds. First, how plausible is it that *townsendi* mtDNA simply mutated to the *virens* genotype? The answer requires an evaluation of the number of mutations that would be necessary to move from the haplotype of one of these species to the other. Note that one of the *townsendi* haplotypes is the same as that for *occidentalis* (Table 3). This polymorphism is restricted to coastal regions of the Pacific Northwest and apparently exists because these two species hybridize and *townsendi* is competitively superior to *occidentalis*. Geographic variation in their mtDNA will be the subject of another paper and need not be considered for this Rocky Mountain specimen (Rohwer, Bermingham and Wood unpubl. data). There is important variation for *Xba* I, where *townsendi* is polymorphic for two haplotypes. One requires two losses and the other three losses to move by mutation from the haplotype of *townsendi* to the haplotype of *virens*. For the last relevant restriction enzyme, *Bam* HI, one loss and one gain would be required to move by mutation from the *townsendi* to the *virens* haplotype. Thus, with a survey of only two relevant restriction enzymes, at least four mutations (three losses and one gain) are required to account for the mtDNA haplotype of this bird through mutation rather than through a hybrid ancestry. Hybrid ancestry is more plausible.

Another possibility is that *townsendi* is polymorphic for both of these cutting sites and that this specimen exhibits a haplotype that is shared between *virens* and *townsendi*. This proposition is highly unlikely for two reasons. First, the specimen in question was collected in an area where the ranges of *virens* and *townsendi* almost make contact, making a hybrid origin a reasonable possibility (see Godfrey 1966). Second, Rohwer, Bermingham and Wood (unpubl. data) have surveyed over 400 *townsendi* from Alaska, British Columbia and Washington; no other *townsendi* specimen carried this *virens* haplotype. There was limited variation involving *Xba* I, but mutants were always just one step removed from the predominant *townsendi* haplotype. In contrast, the individual in question was three losses and one gain removed from the predominant *townsendi* haplotype considering both *Xba* I and *Bam* HI.

Range and habitat.—The *townsendi* × *nigrescens* hybrid was collected about midway up the west side of Vancouver Island near Gold River. In several summers of extensive fieldwork collecting *townsendi, occidentalis* and their hybrids in Canada and Washington, this is one of only a few localities where we have found *townsendi* and *nigrescens* in syntopy. In western Oregon, *nigrescens* and *occidentalis* are found in syntopy in mixed oak (*Quercus garrayna*) and Douglas fir forests (Morrison 1982); in western Washington, they are syntopic in mixed alder (*Alnus rubra*) and fir forests (pers. obs.). In both Oregon and Washington, *occidentalis* preferentially forages in firs, whereas *nigrescens* prefers alders or oaks (Morrison 1982; pers. obs).

In Washington, *townsendi* is rarely syntopic with *nigrescens* (I have found it so only at two localities); *townsendi* is found in fir forests at higher elevations than *nigrescens* usually occurs. This situation was dramatically different on the west side of Vancouver Island, where *occidentalis* is absent. There, I found *townsendi* at sea level and absent in higher-elevation forests. In these extremely wet coastal forests, slight gains in elevation produce forests of cedar (*Thuja plicata*) and hemlock (*Tsuga heterophylla*), neither of which is used by any of these three warblers. Thus, the region in which the *townsendi* × *nigrescens* hybrid was collected is not only at the northern limit of the range of *nigrescens* (Godfrey 1966) but, coincidentally, is also in a region where *nigrescens* and *townsendi* are syntopic as a consequence of *townsendi* being restricted in coastal areas to lowland forests where Douglas firs grow.

Vancouver Island has been so extensively logged that mature fir forests were extremely difficult to find in the vicinity of Gold River. It is perhaps not coincidental that the hybrid specimen was collected about 16 km inland from where the two species were syntopic. It was raining when the specimen was collected and no warblers were singing. My assistant and I had driven some distance inland from the sea level head of Muchalat Inlet, and run into forests of cedar and hemlock. In desperation, I played a tape of *townsendi* songs at a second-growth plantation of Douglas fir that looked much too dense and young (trees less than 8 m high) to be suitable habitat. The warbler responded promptly. Numerous additional playbacks at other places in this stand produced no other warblers, nor did several playbacks in nearby mature cedar–hemlock forests. Near the town of Gold River, *townsendi* was reasonably common in what few stands of Douglas fir could be located; only a single *nigrescens* was seen or heard singing, it being attracted to a *townsendi* playback that netted a *townsendi* specimen in a mixed stand of alder and Douglas fir. Thus, it seems more than coincidental that this hybrid was taken in an area where *nigrescens* was rare and where the range of *townsendi* descends to sea level. Furthermore, it was taken in extremely marginal habitat some distance from areas where the two parental forms were found together in mixed fir and alder forests. Perhaps as a hybrid it had failed to compete successfully with either parental type.

DISCUSSION

One purpose of this paper has been to introduce new methodology for the identification of hybrids. Mitochondrial-DNA haplotypes can suggest interbreeding that took place long enough in the past for backcrossing to have eliminated all evidence of a hybrid origin from the phenotype. Seriously entertaining such a possibility, however, requires excluding the possibility of a retained ancestral polymorphism in the paternal lineage. In several hundred *townsendi* specimens surveyed from Alaska, British Columbia and Washington, no other evidence was found for a haplotype polymorphism in *townsendi* that included a *virens* haplotype (Rohwer, Bermingham and Wood unpubl. data). In this context, finding a *townsendi* specimen carrying a *virens* haplotype becomes rather convincing evidence that the specimen was of hybrid origin.

In analyzing the *townsendi* × *nigrescens* hybrid, I have illustrated the value of contradictory character states in eliminating alternative pairs of potential parents. Intermediate character states have always been used in analyses of hybrids, and most authors also provide information about how they excluded other possible parents. What is new here is my summary table that collapses information on intermediate and contradictory characters into a single matrix that quantifies the basis for conclusions. I also use this example to demonstrate the fact that only contradictory character states can exclude hypothetical parents that would be acceptable on morphological grounds.

General reviews of the relative frequency of intra- versus intergeneric hybridization for manakins (Pipridae; Parkes 1961), hummingbirds (Trochilidae; Banks and Johnson 1961) and wood-warblers (Parulidae; Parkes 1978, Bledsoe 1988, Morse 1989) have revealed that hybrids between species in different genera are more frequent than hybrids between congeners. Three hypotheses have been proposed to explain this phenomenon. Short (1969) and coworkers (Short and Phillips 1966, Short and Robbins 1967) argued that this phenomenon is a consequence of generic oversplitting. However, systematic revision would only redefine the problem semantically because, even if the generic limits in these families were revised, more of the hybrids would be between distantly related than between closely related species. Thus, the problem would remain.

Banks and Johnson (1961) and Parkes (1961, 1978) independently have offered another solution, namely that selection favors isolating mechanisms only between closely related and sympatric species, and not between more distantly related species or species that are rarely in contact. In fact, a number of the intergeneric warbler hybrids and most of the intergeneric hummingbird hybrids are between species whose ranges barely make contact (Morse 1989, Banks and Johnson 1961).

In view of this hypothesis, it is interesting to consider the two new *Dendroica* hybrids reported here in the context of a phylogeny for the *virens* species group based on mtDNA restriction sites (Bermingham et al. 1992). The split between *nigrescens* and the clade including *virens, townsendi* and *occidentalis* is basal and may date to the Pliocene; the split between *townsendi* and *occidentalis* is quite recent (Bermingham et al. 1992). Unfortunately, tissues of *chrysoparia* were unavailable for this phylogenetic analysis. Since the split between the *nigrescens* and *virens* clades is old, the new hybrid between *nigrescens* and *townsendi* is yet another case of rather distantly related species producing a hybrid. As I reported above, although these species exhibit broad geographic sympatry, the syntopy observed near the collecting locality for this hybrid is unusual. In contrast, syntopy between the more closely related pair, *occidentalis* and *nigrescens,* is common in Washington and Oregon (Morrison 1982), but no hybrids between them are known.

The ranges of *virens* and *townsendi* are not even known to make contact. Thus, although they are more closely related than *nigrescens* and *townsendi,* the potential for hybrid pairings between *virens* and *townsendi* is, under present-day distributions, very low.

Short (1969) reviewed evidence compatible with an alternative to the hypothesis that selection has operated to improve reproductive isolation only between closely related species in sympatry. Hybrid pairings often are frequent when rarity of one of the parental species creates a shortage of conspecific mates. This is compatible with intergeneric hybrids often being found in warblers and hummingbirds at the edges of species ranges (Banks and Johnson 1961, Parkes 1978). Both of my new hybrids may fit this hypothesis. *Dendroica nigrescens* is at the very limit of its range where I collected the *nigrescens* × *townsendi* hybrid on Vancouver Island, and it is easy to imagine a female having difficulty finding a mate there because *nigrescens* is uncommon on Vancouver Island. Breeding of *virens* has not been recorded quite as far west in British Columbia as Valemount, where we collected the *townsendi* specimen carrying an mtDNA haplotype of *virens.*

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LITERATURE CITED

BANKS, R. C., AND N. K. JOHNSON. 1961. A review of North American hybrid hummingbirds. Condor 63:3–28.

BERMINGHAM, E., S. ROHWER, S. FREEMAN, AND C. WOOD. 1992. Vicarance biogeography in the Pleistocene and speciation in North American wood warblers: A test of Mengel's model. Proc. Natl. Acad. Sci. USA 89:6624–6628.

BLEDSOE, A. H. 1988. A hybrid *Oporornis philadelphia* × *Geothlypis trichas,* with comments on the taxonomic interpretation and evolutionary significance of intergeneric hybridization. Wilson Bull. 100:1–8.

CRACRAFT, J. C. 1983. Species concepts and speciation analysis. Curr. Ornithol. 1:159–187.

GODFREY, W. E. 1966. The birds of Canada. National Museum of Canada, Bull. 203.

JACKSON, W. M., C. S. WOOD, AND S. A. ROHWER. 1992. Age-specific plumage characters and annual molt schedules of Hermit and Townsend's warblers. Condor 94:490–501.

MCKITRICK, M. C., AND R. M. ZINK. 1988. Species concepts in ornithology. Condor 90:1–14.

MENGEL, R. M. 1964. The probable history of species formation in some northern wood warblers (Parulidae). Living Bird 3:9–43.

MENGEL, R. M. 1970. The North American central plains as an isolating agent in bird speciation. Univ. Kansas, Dep. Geology, Spec. Publ. 3:279–340.

MORRISON, M. L. 1982. The structure of western warbler assemblages: Ecomorphological analysis of the Black-throated Gray and Hermit warblers. Auk 99:503–513.

MORSE, D. H. 1989. American warblers: An ecological and behavioral perspective. Harvard Univ. Press, Cambridge, Massachusetts.

PARKES, K. C. 1961. Intergeneric hybrids in the family Pipridae. Condor 63:345–350.

PARKES, K. C. 1978. Still another parulid intergeneric hybrid (*Mniotilta* × *Dendroica*) and its taxonomic and evolutionary implications. Auk 95:682–690.

PYLE, P., S. N. G. HOWELL, R. P. YUNICK, AND D. F. DESANTE. 1987. Identification guide to North American passerines. Slate Creek Press, Bolinas, California.

ROHWER, S. A. 1972. A multivariate assessment of interbreeding between the meadowlarks, *Sturnella*. Syst. Zool. 21:313–338.

ROSEN, D. E. 1979. Fishes from the uplands and intermontane basins of Guatemala: Revisionary studies and comparative geography. Bull. Am. Mus. Nat. Hist. 162:267–376.

SHIELDS, G. F., AND K. M. HELM-BYCHOWSKI. 1988. Mitochondrial DNA of birds. Curr. Ornithol. 5:273–295.

SHORT, L. L. 1969. Taxonomic aspects of avian hybridization. Auk 86:84–105.

SHORT, L. L., JR., AND A. R. PHILLIPS. 1966. More hybrid hummingbirds from the United States. Auk 83:253–265.

SHORT, L. L., JR., AND C. S. ROBBINS. 1967. An intergeneric hybrid wood warbler (*Seiurus* × *Dendroica*). Auk 84:534–543.