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Variation and zoogeography of the turtle *Chrysemys picta* in Virginia, USA

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ABSTRACT - During research to determine the subspecific status of the painted turtles, *Chrysemys picta*, and their distribution in Virginia, USA, 1082 turtles from 12 watersheds and 68 counties and municipalities were examined. Watersheds studied included the eastern flowing Shenandoah/Potomac (**A**), Rappahanock/Rapidan (**B**), York (**C**), and James (**D**); the southward flowing Dismal Swamp (**E**), Chowan (**F**), Roanoke (**G**), and Pee Dee (**H**); the small waterways of the Delmarva Eastern Shore (**I**) flowing west into the Chesapeake Bay or east into the Atlantic Ocean; the New River (**J**) that flows northward from North Carolina through western Virginia; and the Tennessee (**K**) and Big Sandy (**L**) watersheds that flow west into the Ohio River, and thence to the Mississippi River.

Two subspecies of painted turtles occur in the Commonwealth, *C. picta picta* [P] and *C. p. marginata* [M]. The 75% Rule of Amadon (1949) was applied to determine if populations of pure P & M exist in Virginia. This revealed that most individuals in the Commonwealth are intergrade (hybrid) MxP. The frequencies of occurrence of P, M, & MxP in these watersheds show that P is predominately a Coastal Plain resident, with a 75% occurrence and a 22% Piedmont occurrence in the Shenandoah/Potomac; 74% Coastal Plain and 36% in the Piedmont of the Rappahannock/Rapidan; and 34% in the Coastal Plain and 64% in the Piedmont of the James. M is essentially restricted to the Piedmont and Headwaters of these watersheds, with only 11% occurring in the Coastal Plain of A. MxP is found in all three physiographic regions of A-D, but in the greatest numbers in the Highlands. This is also true in the Piedmont of the southflowing rivers F-H. Turtles from the western flowing K & L are predominately MxP with lesser numbers of M. The possible origins of these taxa in Virginia's waterways is discussed and compared with the hypothesis of the zoogeographical distribution of North Americas *C. picta* of Bleakney (1958), and the results and conclusions of Ultsch et al. (2001) who considered P to not exist in pure form because of intergradations (hybridization) with M along its south-north distribution.

INTRODUCTION

The painted turtle, *Chrysemys picta*, is the only North American freshwater turtle whose natural range extends from the Atlantic to the Pacific coasts (Ernst & Lovich, 2009). Such a great distribution has led to phenotypic color pattern and morphological variation, resulting in four subspecies being described (Ernst, 1971).

Serious study of variation in *C. picta* was begun by Bishop & Schmidt (1931) and Hartweg (1934); and since has led to a number of regional studies showing intergradation (hybridization) between the four subspecies (Ernst & Lovich, 2009). Such studies have been reported from the Mid-Atlantic states of Pennsylvania (Ernst & Ernst, 1971) and Maryland (Groves, 1983) to the north, the Gulf states to the south (Ernst, 1967), and Kentucky and Tennessee to the west of Virginia (Ernst, 1970; Johnson, 1954).

C. picta has been in Virginia at least since the Late Quaternary (Holman & McDonald, 1986); and two possible subspecies occur in the Commonwealth (Figs. 2-3); the eastern painted turtle, *Chrysemys picta picta* (P), and the midland painted turtle, *Chrysemys picta marginata* (M). P has a maximum straight line carapace length (SCLmax) of 19.0 cm, its vertebral and pleural carapace scutes <30% disaligned, light (yellow, orange, red or a blending of these colours) borders along the carapacial seams (>2.5mm bordering the pleural seams), a narrow (>1.5 mm) continuous mid-dorsal carapace stripe, and an unmarked (Fig. 2) yellow or only lightly dark spotted plastron (Bishop & Schmidt, 1931). M has a SCLmax of 19.5 cm, alternate vertebral and pleural carapace seams (>30% disalignment), narrow (<2.5mm), dark (black, olive or none) bordered carapacial seams, an absent or poorly developed (discontinuous), narrow (<1.5mm) mid-dorsal carapace stripe, and a plastron marked (Figs. 3-4) with a variable dark central figure (Bishop & Schmidt, 1931). Intergrades (MxP) have a majority of these characters intermediate between P and M. To accurately determine the status of individual turtles, "all" of the above characters must be considered; not only the percentage of seam disalignment and the plastron pattern.

Mitchell (1994) reported that "considerable variation exists throughout Virginia [*C. picta*] populations in the two characters that distinguish *C. p. picta*...from *C. p. marginata*... the alignment of the seams on the pleural and vertebral scutes, and the presence of the marginata type of figure on the plastron... However, only in the upper James River drainage in Bath and Highland counties could Virginia populations be considered intergrades between the two subspecies...

Intergrades may also occur in extreme northern Virginia (C. H. Ernst, pers. comm.).” Although Mitchell (1994) has reported that some turtles seem to be intergrades between eastern P and the more western M, only P has been documented from Virginia (Mitchell & Reay, 1999; Tobey, 1985). In spite of these conclusions, no formal study of intergradation between P and M in Virginia has been reported until now. Herein are the results of an extensive study to determine the variation, taxonomic status, and zoogeography of Virginia’s painted turtles.

While the concept of subspecies is controversial, there is utility in its application for zoogeographical and conservation studies (Haig et al., 2006). However, standards on which a subspecies is determined have been vague and variously used. The only quantitative metric that we have found is the 75% Rule of Amadon (1949; as discussed by Patten & Unitt, 2002) that 75% of the individuals in the population in question must be distinguishable from all those from the most proximate populations to be considered a unique subspecies. In this case the Virginia painted turtles versus the populations of P and M in adjacent states; our analysis of the subspecific status of painted turtle populations in the major Virginia watersheds is based on this principle.

METHODS

All turtles were sexed by the characters listed in Ernst (1971). Scute terminology is that of Ernst & Lovich (2009). The SCLmax and greatest nonmedial plastron length (PL) of each turtle were recorded. All measurements were made with metric dial calipers accurate to 0.1 mm.

The methods of measurement introduced by Hartman (1958) were used to compare the degree of disalignment of the carapace scutes. When the seams between the central vertebral scutes and lateral pleural scutes lie in the same transverse line they were considered to be 0% disaligned; if the seams alternate exactly they were considered 100% disaligned. The base point for measuring is the inner end of the seam separating the second and third pleural scutes. The imaginary line from the base point forward and parallel to the longitudinal axis of the carapace, to the point opposite the inner end of the seam between the second and third pleurals was measured, and was denoted as 1a on the left side and 2a on the right side. The part of this same imaginary line ending at the base point between the second and third vertebrals was measured and denoted 1b on the left side and 2b on the right side (See Fig. 2 of Hartman, 1958.). The average percent disalignment was then calculated as 1b/1a + 2b/2a. P exhibits none or very low disalignment, normally <30%; while M has disalignment >30%.

The light border of the posterior seam of the second pleural was measured at its widest point and its colour noted. Red, orange or yellow borders were considered characters of P; black, olive, or no border pigmentation were considered those of M (Ernst & Ernst, 1971). The greatest width of the mid-dorsal stripe (CDS) on the carapace was measured on the second vertebral scute. M usually has a discontinuous mid-dorsal stripe while that of P is normally uninterrupted. Completeness of the CDS was calculated as a percentage of the greatest width of the broadest foreleg stripe (FLS), CDS/FLS (P >70%, M <50, MxP 51-69%). The extent of plastron pigmentation as described above was illustrated in Bishop & Schmidt (1931).

All data were statistically analyzed using SAS package 8.2; levels of significance were set a priori at $\alpha = 0.05$. To compare possible intergrades (hybrids), Fisher’s discriminant analysis was run on combined data using the following nonsexually dimorphic variables: pleural scute disalignment, seam width, central stripe width, and the width of the widest foreleg stripe. Normality was checked visually using probability plots. To analyze the data, first, a training data set was created using data from known pure taxa from Kentucky, Maryland, North Carolina, Pennsylvania, and Virginia (Ernst, 1970; Ernst & Ernst, 1971; Ernst et al., 2006; Groves, 1983; Ultsch et al., 2001).

Study Areas. *C. picta* (N=1082) from 12 river watersheds (Fig. 1) and 68 counties and municipalities in Virginia were examined. The western Appalachian Mountains divide its river drainages; those flowing east or south eventually enter the Atlantic Ocean; those flowing west enter the Gulf of Mexico (Jenkins & Burkhead, 1994).

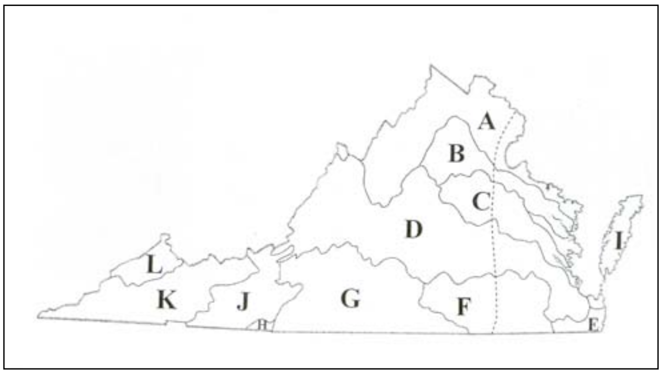

Figure 1. Virginia watersheds from which specimens of *C. picta* were studied: A, Shenandoah/Potomac; B, Rappahanock/Rapidan; C, York; D, James; E, Dismal Swamp; F, Chowan; G. Roanoke; H, Pee Dee; I, Delmarva Eastern Shore; J, New; K, Tennessee; and L, Big Sandy. Dashed north-south line denotes the Fall Line separating the Coastal Plain from the Piedmont and Western Headwaters.

Several major river watersheds eventually flow eastward toward the Chesapeake Bay and Atlantic Ocean; from north to south these are: The Potomac/Shenandoah Drainage [A] drains about 39% of Virginia with tributaries both west and east of the Blue Ridge Mountains, and eventually crosses the Fall Line (the upstream limit for commercial boating traffic) at Great Falls, Fairfax County, and flows into the Chesapeake Bay. It also receives water from West Virginia, Maryland, and Pennsylvania. Ernst et al. (1997) reported the painted turtles from the vicinity of Fairfax County as intergrade MxP. The Rappahannock/Rapidan Drainage [B] occurs entirely within Virginia. The Rappahannock begins in the Blue Ridge Mountains and flows eastward through the Piedmont of central Virginia to join the mostly south flowing Rapidan River near the Fall Line at Fredericksburg and finally empties into the Chesapeake Bay. The York River [C] results from the jointure of the Mattaponi River and Pamunkey Rivers (formed by the union of the North and South Anna rivers), and is essentially a Coastal Plain drainage entering the Chesapeake Bay between [B] and the James River [D]. The James River watershed [D], with the exception of two streams originating

Figure 2. *C. picta picta*: carapace, Roger W. Barbour; plastron, Richard D. Bartlett.

Figure 3. *C. picta marginata*: carapace and plastron, Roger W. Barbour.

in West Virginia, flows east entirely through Virginia, draining almost 25% of the Commonwealth, and finally enters the Chesapeake Bay. It crosses the Fall Line east of the Richmond Basin. Although Mitchell (1994) reported that only painted turtles from Bath and Highland counties in the headwaters of this watershed are MxP, Schwab (1989) described a turtle from Prince George County near its mouth that was clearly a MxP.

Several other drainages flow southward into North Carolina and finally empty into the Atlantic Ocean: The Dismal Swamp watershed [E] generally flows south in the Coastal Plain from extreme southeastern Virginia into North Carolina and empties into the Albemarle Sound. Pague & Mitchell (1991) reported P present at Back Bay northeast of the Great Dismal Swamp. Southeastern Virginia's Chowan River [F] is made up of the Nottaway and Meherrin rivers from the Piedmont above the fall line and the Blackwater River of the Coastal Plain. It enters northeastern North Carolina and finally flows into the Albemarle Sound. The Roanoke River watershed [G] occurs above the Fall Line in southcentral Virginia, flows southeastward draining about 16% of Virginia into North Carolina, and eventually reaches the Albemarle Sound west of the Chowan River (which is sometimes considered part of the Roanoke system). The Pee Dee watershed [H] is essentially a North and South Carolina system with only a small portion in Virginia. It arises as the Ararat River in the Blue Ridge Mountains and flows southeastward into the Yadkin River that joins the Pee Dee River in southern North Carolina which finally meets the Atlantic Ocean in South Carolina.

The southern Delmarva Eastern Shore watershed [I] is composed of short Coastal Plain waterways in Accomack and Northampton counties that flow either west into the Chesapeake Bay or east into the Atlantic Ocean. P has been reported from Assateague and Chincoteague islands (Lee, 1972; Mitchell & Anderson, 1994; where they were apparently introduced [W.A. Dunson, pers. comm., in Mitchell & Anderson, 1994].

The major Virginia watersheds flowing westward to the Gulf of Mexico via the Ohio and Mississippi rivers are as follows. The New River system [J] arises in North Carolina, flows northward through southwestern Virginia's southern Blue Ridge Highlands and more northern Valley and Ridge physiographic provinces, crosses much of West Virginia, and finally enters the Ohio River drainage. Unfortunately, the only specimen from the New River examined was a DOR individual from Floyd County consisting of shell pieces with no identifiable taxonomic characters (Virginia Museum of Natural History [VMNH] 150006). Seidel (1981 [1982]), during a study of the species of Pseudemys in the river, reported the painted turtles there to be intergrade MxP. The Clinch and Holston rivers originate in southwestern Virginia and flowing westward, form the headwaters of the Tennessee River system [K], a major tributary of the Ohio River. The Big Sandy River watershed [L] begins in the Appalachian Plateau of southwestern Virginia and forms the border between Kentucky and West Virginia while flowing northward to the Ohio River.

		Subspecific Character					
		Carapace Seam	Carapace Dorsal Stripe				
Watershed (N)	Plastron Pattern (%)	Disalignment (%)	Colour (%)	Width (mm)	Condition (%)	Width (mm)	CDS/FLS (%)
A (199)	P 104 (52.3)	23.4	Y 121 (60.8)	2.81	Cont. 131 (65.8)	1.0	70.8
	M 57 (28.6)	(4.7-62.2)	R 2 (1.0)	(0.1-9.8)	Disc. 68(34.2)	(0.4-34.2)	(22.2-131.8)
	MxP 38 (19.1)		RY 69 (34.7)				
			OR 3 (1.5)				
			Ol 2(1.0)				
			Or 1(0.5)				
			OlY 1 (0.5)				
B (28)	P 21 (75.0)	18.7	Y 25 (89.3)	2.98	Cont. 23 (82.1)	1.2	52.3
	M 3 (10.7)	(1.2-55.6)	RY 3 (10.7)	(0.5-4.6)	Disc. 5 (17.9)	(0-4)	(0-100)
	MxP 4 (14.3)						
C (16)	P 9 (56.3)	20.2	Y 15 (93.8)	3.27	Cont. 11 (68.8)	0.9	58.6
	M 3 (18.8)	(8.3-53.5)	RY 1 (6.2)	(2.1-5.0)	Disc. 5 (31.3)	(0.6-4.7)	(20-100)
	MxP 4 (25.0)						
D (494)	P 229 (46.4)	22.3	Y 444 (899)	2.80	Cont 356 (72.1)	0.9	61.2
	M 18 (3.6)	(2.0-26.9)	RY 44 (8.9)	(0.1-6.9)	Disc. 138 (27.9)	(0-2)	(0-180)
	MxP 247 (50.0)		Or 3 (0.6)				
			OrY 2 (0.4)				
			OR 1 (0.2)				
E (73)	P 42 (57.5)	20.9	Y 54 (79.0)	2.36	Cont. 57 (78.1)	0.7	58.9
	M 10 (13.7)	(5-50)	RY 12 (16.4)	(0.2-4.5)	Disc. 16 (21.9)	(0-1.2)	(0-120)
	MxP 21 (28.8)		Or 3 (4.1)				
			OrY 4 (5.5)				
F (70)	P 43 (61.4)	18.7	Y 59 (84.3)	2.01	Cont. 56 (80.0)	0.7	59.1
	M 8 (11.4)	(5.0-48.8)	OR 5 (7.1)	(0.2-4.5)	Disc. 14 (20.0)	(0.3-1.6)	(0-109.1)
	MxP 19 (27.1)		R 2 (3.0)				
			RY 2 (3.0)				
			Ol 2 (3.0)				
G (69)	P 29 (42.0)	26.1	Y 57 (82.6)	2.10	Cont. 55 (79.7)	8.4	69.5
	M 22 (31.9)	(7.8-63.9)	Or 4 (5.8)	(0.1-3.9)	Disc. 14 (20.3)	(0.4-1.2)	(36.4-122.2)
	MxP 18 (26.1)		OrY 4 (5.8)				
			R 2 (3.0)				
			RY 2 (3.0)				
H (7)	P 4 (57.1)	13.2	Y 5 (71.4)	1.5	Cont. 5 (71.4)	0.5	55.0
	M 2 (28.6)	(3.4-34.5)	RY 2(28.5)	(0.7-2.7)	Disc. 2 (18.5)	(0.4-0.7)	(33.3-100.0)
	MxP 1 (14.3)						
I (110)	P 61 (55.3)	26.3	Y 60 (54.5)	2.2	Cont. 84 (76.4)	0.8	58.7
	M 10 (9.1)	(0.8-67.5)	R 2 (1.8)	(0.1-4.8)	Disc. 26 (23.6)	(0-4)	(0-175)
	MxP 40 (36.3)		RY 41 (37.3)				
			Ol 7 (6.4)				
J (1*)	-	-	-	-	-	-	-
K (6)	P 1 (16.7)	26.3	Y 6 (100)	1.0	Cont. 5 (83.3)	0.6	27.9
	M 1 (16.7)	(10.9-34.0)		(0.6-2.3)	Disc. 1 (16.7)	(0.3-1.1)	(0-65)
	MxP 4 (66.7)						
L (9)	P 5 (55.6)	22.0	Y 9 (100)	2.5	Cont. 5 (55.6)	0.9	64.7
	M 3 (33.3)	(10.5-36.5)		(0.8-3.7)	Disc. (44.4)	(0.6-1.1)	(42.1-91.7)
	MxP 1 (11.1)						

Table 1. Phenotypic characters by watersheds for Virginia *C. picta* (P = *picta*, M = *marginata*, MxP = *marginata* x *picta* intergrades. Watersheds are described in the text; see Figure 1 for locations. *See text for explanation: CDS = carapace dorsal stripe, FLS = Foreleg stripe. Colours: Y=yellow, R=red, O=orange, Ol=olive.

	Taxon Frequency		
Watershed (N)	***picta***	***marginata***	***marginata* x *picta***
A (199)	72 (36.2)	28 (14.1%)	99 (49.7%)
B (28)	14 (50%)	1 (3.6%)	13 (46.4%)
C (16)	5 (31.3%)	0 (-)	11 (68.8%)
D (494)	229 (46.4%)	18 (3.6%)	247 (50.0%)
E (73)	26 (35.6%)	6 (8.2%)	41 (56.2%)
F (70)	43 (61.4%)	8 (11.4%)	19 (27.1%)
G (69)	15 (14.5%)	8 (11.6%)	46 (66.7%)
H (7)	1 (14.3%)	0 (-)	6 (85.7%)
I (110)	51 (46.4%)	8 (7.3%)	51 (46.4%)
J (1*)	-	-	-
K (6)	0 (-)	1 (20.0%)	5 (80.0%)
L (9)	0	1 (11.1%)	8 (88.9%)
Total (N) 1082	**455 (42.2%)**	**79 (7.3%)**	**545 (50.5%)**

Table 2. Taxonomic subspecific status of *C. picta* (N) examined per Virginia Watershed (see text for explanations; and Figure 1 for locations); *VMNH 150006, Floyd County, DOR specimen consisting of shell pieces with no reliable data, see text.

Watershed (N)	% *picta* (N)	% *marginata* (N)	% *marginata* x *picta* (N)
Potomac/Shenandoah (199)			
Coastal Plain	75.0% (54)	10.7% (3)	13.1% (13)
Piedmont	22.2% (16)	32.1% (9)	17.2% (17)
Highlands	2.8% (2)	57.1% (16)	69.7% (69)
Total (199)	36.2% (72)	14.1% (28)	49.7% (99)
Rappahannock/Rapidan (28)			
Coastal Plain	64.3% (9)	- (0)	7.7% (1)
Piedmont	35.6% (5)	- (0)	42.6% (6)
Headwaters	(0)	100.0% (1)	46.2% (6)
Total (28)	50.0% (14)	3.6% (1)	46.4% (13)
James (494)			
Coastal Plain	34.5% (79)	- (0)	17.4% (43)
Piedmont	65.1% (149)	72.2% (13)	72.5% (179)
Headwaters	0.4% (1)	27.7% (5)	10.1% (25)
Total (494)	46.4% (229)	3.6% (18)	50.0% (247)

Table 3. Distribution of the subspecific taxa in the three major East to West watersheds of Virginia (see text for descriptions). N = numbers of individuals.

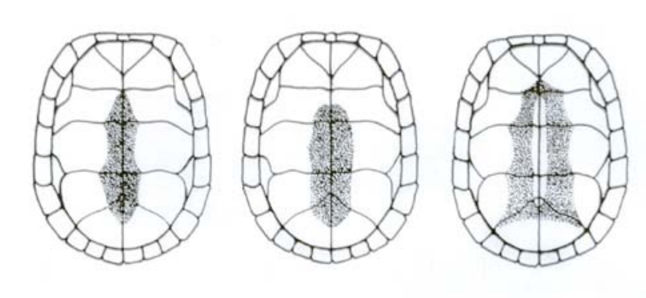

Figure 4. Variations in the plastron pattern of *C. picta marginata* (from Bishop & Schmidt 1931).

RESULTS

Each of the 1082 *C. picta* was examined visually to determine its plastron pattern, carapace seam colour, and the condition of its carapace dorsal stripe (CDS); and measured to determine the percentage of disalignment and width of its carapace seams, and the widths of its carapace dorsal stripe (CDS) and widest foreleg stripe (FLS) to determine its CDS/FLS. These characters (see above) were then used to assign each turtle to a subspecific taxon (P, M, MxP). The tabulated results are presented in Tables 1-3.

Tables 1 & 2 show the subspecific diagnostic characters and the taxon frequencies of each watershed. It is clear from the data in these two tables that only watershed A reaches 70% frequency of P, and that P and M overall amounted to less than 50% and 10% of the 1082 Virginia painted turtles examined. The only exceptions were the Rappahannock/Rapidan with 50% P, and the Chowan with 61.4% P. Although the painted turtles of Virginia have been referred to as P, they are clearly not predominately that subspecies. Instead they form an intergrade (hybrid) swarm of P, M, and MxP, with the great majority of the turtles being MxP (Table 2).

DISCUSSION

Our zoogeographic hypothesis of the distributional origins of Virginia's painted turtles is based on Bleakney (1958), who proposed a theory as to the centers of origin of the four subspecies of *C. picta* and how they subsequently migrated into their present geographical ranges after the Wisconsinian glacial period. He proposed that at the end of the last North American glacial period *C. picta* was divided into three separate populations that represented separate incipient species: *C. picta* in the southeastern Atlantic coastal region, *C. dorsalis* in the lower Mississippi River Valley, and *C. bellii* in the Rio Grande and Pecos river watersheds of New Mexico. He thought that these three populations extended their ranges northward with the final retreat of the glaciers: *C. dorsalis* moved up the Mississippi River and met *C. bellii* in the region of St. Louis "near the Missouri-Mississippi-Ohio [rivers] junctures." There, Bleakney hypothesized the two hybridized and produced the species *C. marginata*. Marginata then migrated up the Mississippi and Ohio river watersheds eventually reaching the northeastern United States and adjacent Canada where they met and interbred with *C. picta*, which had migrated up the Atlantic Coast, and also met with and interbred with *C. bellii* in the area of Wisconsin and Michigan. Because the four "species" of painted turtles had not evolved reproductive isolation to prevent genetic exchange by the time their ranges met, broad zones of intergradation (hybridization) were established (Ernst & Lovich, 2009). However, Ernst et al. (2006) have proposed that the center of origin of *C. marginata*, based on the existing fossil record, was more likely the Tennessee River Valley, from which it spread north and east. Our discussion of the possible zoogeography of the modern subspecies of *C. picta* in Virginia are only concerned with the subspecies M and P.

Painted turtles possibly reached Virginia after the Wisconsinin Ice Age by four ways: directly up or downstream along major existing waterways, stream capture, overland migration between nearby separate drainages, or by translocation by Native Americans who used them for food or in their ceremonies (Adler, 1968; Lovich et al., 2014). Unfortunately, there is no way of determining the role of humans in the introduction and distribution of painted turtles in Virginia. Thus, our research centered on the species current distribution in the 12 watersheds of the Commonwealth.

Genes from M had three possible directions of entry into Virginia's waterways; from what is now northwestern North Carolina; from now Tennessee, Kentucky, and West Virginia to the west; or from present Maryland and Pennsylvania to the north.

The identity of the recent painted turtle colonies in northwestern North Carolina has not been established with certainty. Palmer & Braswell (1995) reported that more specimens are needed from the mountains and Piedmont of western North Carolina to establish the range of M there, and consequently referred to all North Carolina populations only as *C. picta*. As M is known from Tennessee adjacent to North Carolina (Ernst, 1970; Johnson, 1954), it is probable that at least some of the populations in western North Carolina are of this subspecies and could have moved north into southern Virginia. The Virginia drainages possibly affected by migration from western North Carolina are the Powell, Clinch, and Holston of the Tennessee River drainage. It is also possible that an opposite migration from Virginia southward may have established M at some North Carolina localities.

M occurs in the mountains of eastern Tennessee (Ernst, 1970; Gentry, 1956; Johnson 1954); and it or MxP are also known from Laurel, Mason, Bath, and Rowan counties in eastern Kentucky (Ernst, 1970). In Kentucky, Clover Fork and Poor Fork, headwaters of the Cumberland River, and Russell Fork, a headwater of the Big Sandy River, are close to Virginia, and are potential migration routes into the Commonwealth. Green & Pauley (1987) reported P from Mercer and Monroe counties, and M from Greenbrier and Summers counties in southeastern West Virginia; indicating that intergradation between the two subspecies occurs in West Virginia. The potential introduction of genes of M from eastern Tennessee, Kentucky and West Virginia exists at least for Virginia's Powell, Clinch, Holston, New, James, and Potomac/Shenandoah watersheds; and Hoffman (1949) reported MxP from Virginia's James River watershed in Monroe County.

To the North, M may have entered Virginia from Maryland to the Potomac River via the Youghiogheny River of the Ohio River watershed west of the Atlantic-Ohio Divide in Pennsylvania, and possibly from West Virginia by stream capture or overland migration (Ernst & Ernst 1971, Groves 1983). It could also have reached the Potomac by moving southward along its tributaries from Adams and Franklin counties, Pennsylvania. Once M reached Virginia, it could have easily migrated southward up the Shenandoah River's branches into westcentral Virginia. Also, the Susquehanna River watershed could have provided M or MxP a more eastern avenue of entry into Virginia via the Chesapeake Bay. The major Virginia watersheds affected by such northern entry paths would be the Potomac/Shenandoah, Rappahannock/Rapidan, York, James Rivers, and those of the Delmarva Eastern Shore.

P would have moved north into Virginia from North Carolina; either up the New River Valley, or upstream in the Pee Dee, Dan, Roanoke, Meherrin, Chowan, or Dismal Swamp drainages, where it eventually met M from the North. P probably reached the Virginia Eastern Shore by migration north along the Chesapeake Bay.

Table 3 shows the physiographic distribution of P, M, and MxP in the three major west-east flowing watersheds (Potomac/Shenandoah[A], Rappahannock/Rapidan[B], and James[D]: the eastern Coastal Plain below the fall line), the central Piedmont above the fall line, and the western Headwaters. Except in the James River, P is found predominately on the Coastal Plain and M on the central and western regions. MxP is found predominately west of the fall line but is also present on the Coastal Plain. This supports the hypotheses of P's northward movement into these three watersheds, and also those of the York [C] and Delmarva/Eastern Shore [I] (Table 2); and the eastern and southward movements of M into Virginia. The taxon frequencies shown in Table 2 of the southward flowing Dismal Swamp [E], Chowan [F], and Roanoke [G], and the north flowing Pee Dee [H] river support a northward flow of P's genes into Virginia from North Carolina.

Although examination of only 15 turtles from the western flowing Tennessee (6) and Big Sandy (9) watersheds revealed no P and only M and MxP, it indicates a western invasion of M.

Our overall results support both Bleakney's (1958) hypothesis of the northward movement of P and the eastward and southward movement of M. They also possibly support the statement by Ultsch et al. (2001) that "pure picta [P] do not exist; there is marginata [M] [genetic] influence throughout picta's range, particularly at the extremes." However, Ultsch et al. (2001) used slightly different subspecific characters and statistical ranges, and had relatively small sample sizes from their various P sites (with the exception of Groton, New London, Connecticut). The major difference between the two studies is that Ultsch et al. (2001) did not apply the 75% subspecific standard (Amadon, 1949). If they had, their results and conclusions may have been different.

Their other confusing conclusion is that P is a genetic intergrade (hybrid) with M, and is thus MxP. They accepted Bleakney's (1958) explanation that M was formed as a result of hybridization between the then *C. bellii* and *C. dorsalis* (see above). This would result in the evolution of an intergrade (hybrid) from a previous intergrade (hybrid) *C. bellii* and *C. dorsalis*. In our opinion, this is a highly unlikely mode of speciation.

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